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MUSEUMS AND ART GALLERIES AS CULTURAL CENTRES

FOR many years the Museums Association has in one way and another been pressing for some form of Government assistance to help, and even in some cases to preserve from extinction, the many small society and trustee museums in Britain. From time to time various organizations have stressed the importance and urgency of the claims of museums and art galleries and recently the Bow Group prepared a detailed memorandum on the subject*.

The main part of this report naturally surveys the facts regarding the present position of the museum and art gallery service. It is pointed out that this is complex and that the official relationships between the national and provincial institutions are thoroughly unsatisfactory. With the increasing leisure of many people and the great advances in organized education made in recent years, it is indeed imperative that museums and art galleries should be in a far better position than they are to deal with not only the preservation of their buildings and contents but also the wider problems of visual education.

The memorandum clearly indicates the complexity of organization in the museum and art gallery field, and points out that some are financed direct from the Treasury, others from the Ministry of Education, some from county or municipal rates and yet others from universities and various societies and trustees.

The fundamental aims of museums and art galleries are discussed, and in addition it is rightly stated that each institution should be a cultural centre to its community, where people may meet their friends in the context of scientific and beautiful objects and where amateur activities can be pursued under favourable conditions.

The report cites outstanding examples of what is urgently required, including the rebuilding of the damaged British Museum galleries, the raising of the purchase grant of the National, Tate and other art galleries, the provision of museums and art galleries in areas where the inhabitants have no ready access to such institutions, and the adequate financing of several now threatened with closure.

The survey naturally leads to recommendations, and emphasizes the need for the Government to realize that such a problem exists and that its very complexity demands a solution at the national, rather than the local, level. In order to achieve a flourishing and effective museums service it is recommended that a Royal Commission should be appointed at once and that some form of central advisory body should be established to integrate the work of the national and provincial museums. This could be done either by extending the powers and membership of the Standing Commission, or by the Ministry of Education, or by setting up an organization comparable to the University Grants Committee. Such a body should have at its disposal a block grant for distribution to individual museums, and in this connexion it is suggested that the grants should be made on the recommendation of regional museum bodies.

The memorandum issued over the signatures of Mr. Richard Carless and Miss Susan le Roux includes an appendix on the importance of the collections and work of the National Gallery, expressing the hope that it will be possible, as indeed it is eminently desirable, to secure the Hampton site for this great national institution.

The main theme of the recommendations of this important memorandum is in complete accord with that of other organizations vitally interested in the future of the museums and art galleries of Great Britain, and it is hoped that the Government will take heed of the unanimity of these representations.

* The Bow Group. Memorandum on the Museum and Art Gallery Service, with special reference to the National Gallery and the Hampton Site question. Pp. 14. (London: The Bow Group, 1958.) 1*s*.

INTELLECTUAL MYSTICISM

The Manifold and the One

By Agnes Arber. Pp. xiii + 146. (London: John Murray (Publishers), Ltd., 1957.) 18*s*. net.

AGNES ARBER'S book is a study of the problem of the One and the Many from the standpoints of philosophy and of mysticism, and it is also a record of a personal quest continued over a long period. This personal note, though sternly restrained, gives an interest to her writing which carries the reader on through the somewhat compressed material which she has brought together from an amazing variety of sources. She makes no secret of her involvement in the problem. "I still remember vividly the glow of emotion aroused in me by the idea—which I discovered inside myself and treasured as my very own—that all such qualities as justice, love, and truth, are in reality *one*", she writes at the beginning of her book, and the "glow" appears again in her brief summing-up at the end.

Brushing aside as irrelevant the discussions of the Logical Positivists and the Semanticists, she considers the systems of metaphysicians from Anaxagoras onwards, in the conviction that most of them are based, consciously or unconsciously, on an intuition of the ultimate unity of the manifold or "the craving to see the universe *sub specie unitatis*". There is, in her view, a close affinity between the philosophical search for Reality and the mystic's search for union with the One. It is, of course, obvious that many systems end in a mystery which is not just a blank wall, but a Reality which is apprehended but not comprehended. In the survey of the mystics, which

forms the most valuable contribution of her study and embraces both eastern and western examples, she gives the greatest weight to those that are least emotional and most intellectual. The essence of the mystical experience is "direct and unmediated contemplation characterized by a peculiarly intense awareness of a Whole as the Unity of all things".

It is difficult to convey in a review the quality of this book which, by reason of the rich material on which the writer works, calls for comment on almost every page. Probably only those who have themselves experienced the nostalgia for unity will be wholehearted students, but even those who are strangers to this 'intuition' can learn much from it about an important tendency in human thought and emotion through the ages. The Christian theologian will have his reservations and questions, particularly on the place of values in the Unity of the Manifold which, in the author's view, is the ultimate Reality. The distinctions of 'good' and 'evil', we are told, on this, the highest, plane vanish. The lines of Rumi—"The man of God is beyond fidelity and faith, To the man of God right and wrong are alike"—are quoted with approval. Why, one might ask, is the mystic way largely a way of 'purification', if, in the end, the One who is the end of that way is indifferent to all human values? Perhaps we may see here why the Christian Church has been somewhat cautious about mysticism, and if this book has a defect, it is that it is too heavily weighted on the intellectual side. At any rate many, who have loved God and experienced union with Him, have loved Him because they believed Him to be the supreme Good and the enemy of evil. For such persons the beginning of their pilgrimage is to range themselves on His side. They have their right to be heard.

This is a valuable work and a learned one. It deserves to be read and pondered.

W. R. Matthews

DUST, SMOKE AND MIST

Particulate Clouds

Dusts, Smokes and Mists—Their Physics and Physical Chemistry and Industrial and Environmental Aspects. By H. L. Green and W. R. Lane. (Spon's General and Industrial Chemistry Series.) Pp. xix+425+8 plates. (London: E. and F. N. Spon, Ltd., 1957.) 70*s*. net.

IMPLEMENTATION of the Clean Air Act requires control of the emission of dust and smoke from furnaces. This is partly achieved by the mode of operation of the process and partly by the collection of particulate material from the flue gases. The methods of collection that are available include gravitational settling, inertial separation, scrubbing, electrostatic precipitation, sonic and ultrasonic agglomeration. The variety of these methods illustrates the many mechanisms that operate in a particulate cloud. Physicists and chemists have contributed much to understanding of clouds during the past decade, following on the pioneer work of Gibbs, Patterson, Whytlaw-Gray, Drinker, Hatch and others. Notable contributions have been made by the Chemical Defence Experimental Establishment at Porton Down, and it is not unexpected to find that, in a book from this school, the authors have rightly attempted a unified treatment of the whole subject.

The task was by no means simple for, as the four pages of literature abbreviations show, relevant work has been published in a heterogeneous set of journals. Those venturing into this field of colloid science for the first time might well be glad of the further warning that the quality of the literature is uneven. The selected references given at the end of each chapter are, therefore, particularly valuable.

In the discussion of the physics and physical chemistry of clouds in the eight chapters of Part I, the essential instability of a cloud, owing to the mechanisms whereby the constituent particles can either grow or shrink, is clearly presented. Following chapters on the production of clouds and their physical characteristics, there is an interesting account of their optical properties. The basic electromagnetic theory has long been known, and, now that the necessary computations have been carried out, experimental data can be interpreted adequately. There follows a long account of coagulation, one of the most important properties of a cloud; this chapter is fundamental to a proper understanding. The next two chapters on deposition and filtration and on sampling and estimation give descriptions of the many ingenious devices that have been developed, dependent on such varied mechanisms as impaction on an obstacle in a flowing stream, thermal and electrostatic precipitation. Lastly, there is an up-to-date discussion of diffusion in the atmosphere.

Industrial and environmental aspects are covered in Part II. Following a brief chapter on collection, accounts are given of health hazards, atmospheric pollution, aerosols in Nature and, lastly, some uses of particulate clouds. These titles indicate the range of phenomena in which clouds have importance, and the treatment illustrates the authors' thesis that many different types of cloud have essentially the same properties. Thus, the theory of impaction of particles on obstacles is applied in the icing of aircraft, the filtration of dusts and smokes, the capture of windborne pollen, the growth of raindrops and the application of insecticidal aerosols.

These examples serve as an illustration of the value of classical scientific studies at the present time, not only to elucidate natural phenomena but also to improve the operation of industrial equipment. Newer as well as the older technologies have need of the knowledge of clouds gained in the past thirty years. Scientists and technologists will find in this timely book a worthy introduction.

The book is well printed and is substantial enough to warrant its price.

R. L. Brown

PRODUCTIVITY AND SOCIAL ORGANIZATION

Productivity and Social Organization

The Ahmedabad Experiment—Technical Innovation, Work Organization and Management. By A. K. Rice. Pp. xiii+298. (London: Tavistock Publications, Ltd., 1958.) 35*s*. net.

IMPROVED technology has greatly contributed to the rising level of industrial productivity. But there is a danger in some situations that technological change may neglect the social and psychological needs of the human operator and so reduce substantially the potential rise in output per man-hour. There are

a number of studies of the human implications of technological change. But only recently has a method of analysis been evolved which treats a production system as an organic whole, and enables the social dimension to be designed to meet both human and technological requirements in a systematic manner. This method of analysis has arisen from the concept of a 'socio-technical' system introduced by E. L. Trist and his colleagues, of whom Mr. Rice is one, at the Tavistock Institute of Human Relations. The concept of a socio-technical system, together with the developing theory of social structure and the work of W. R. Bion on the conscious and unconscious behaviour of small groups, lays much of the conceptual foundation for the experiments carried out by Mr. Rice in the operational setting of a large Indian textile firm and described in this book.

Mr. Rice worked as a management consultant, and his primary professional responsibility was toward his client. The fact that his client's share prices doubled in value after his three-year period of work at a time when other textile firms were failing suggests that Mr. Rice's contribution in this role was by no means ineffective. But he also worked in an operational research role. Here he used as his quantitative data the commercial and technical facts of the firm, and as his qualitative data the feelings of people. His findings are logically presented and he offers some challenging hypotheses concerning both technological change and the theory of organization.

Mr. Rice's first task was to examine the working of the automatic loom sheds, where despite the introduction of Western technology, productivity and quality were low. An analysis of the socio-technical system showed that "because the workers formed only an aggregate and not a working group, and because the jobs were broken down into component operations" stable relationships were impossible. Nobody experienced either as an individual or in a group the satisfactions deriving from the "completion of a whole task". The production system was therefore re-organized to provide adequate satisfactions in the social dimension. This was followed by a similar reorganization of the non-automatic loom sheds. Despite many difficulties, some of them peculiar to the Indian scene, productivity and quality rose to the levels required by management, in both sheds.

Mr. Rice's third major task was to examine the management organization of the firm. His theory of the differentiation of organization structure into managing and operating systems represents a radical departure from the outworn 'machine' theory of organization and provides an organic model for the study of organizations. Using this model, which is derived from von Bertalanffy's theory of open systems, it is possible for the first time to determine by means of operational criteria where and at what level management functions such as control and services should be placed in an organization. As Mr. Rice remarks, "difficulties between managers are frequently caused by confusion between control functions and operations", and one can only guess at the loss of both productivity and human satisfaction in almost every firm for this reason alone. This new thinking deserves most serious consideration, and I believe it will prove to be a major contribution to the theory of social organization. If there is any criticism to be made, it is that more detail would have been welcomed on the organizational changes and the difficulties which were incurred in making them. No doubt we shall have to wait for this until another occasion. The fact that these remarkable experiments took place in an Eastern culture demonstrates not the limitations but the wide applicability of this integrated approach to a central problem of our time. This is an important book not only for social scientists but for all who are concerned with the tasks of administration in any field.

S. David M. King

JOLLY GOOD COMPANY

Earth's Company

By Leslie Reid. Pp. xi+221+12 plates. (London: John Murray (Publishers), Ltd., 1958.) 25*s.* net.

WITHIN the last decade a spate of books describing various aspects of biology has been published with the avowed aim of interesting the intelligent layman and setting before him different facets of the great natural panorama. Some of these books, notably in the New Naturalist series, have been very successful, combining interest, accuracy, and a dispassionate approach to scientific problems which are wholly admirable. "Earth's Company" makes an attempt to follow this pattern, but goes off on a rather different tack.

Leslie Reid has put three component parts into his book. It is on the surface a description of many aspects of, mainly animal, ecology such as coloration, prey–predator relationships, parasitism, movement of individuals and societies, courtship, etc., and an attempt is made to show that "each single phenomenon of nature has no meaning, no existence, except by virtue of its relationship with all the other phenomena" (p. 208). The ecological accounts are interesting, but on the whole elementary and rather sketchy. In fact in many places the author apologizes for the summary nature of his treatment; for example, work on behaviour in mammals is discussed in a very vague paragraph of ten lines.

However, if one assumes, as one must, that this book is intended for a lay audience, then these weaknesses may be excused. There are, however, a number of inaccuracies; for example, on p. 34 it is stated that "the dominant plants . . . are always the most numerous" and that "natural mutations are extremely rare" (p. 124). These statements depend on the definition of the dominant plant, for if an oak tree is the dominant in an oak wood then there are certainly many more herbaceous plants than there are oaks, and mutations are only rare if one is thinking in terms of one particular mutation at a certain locus in one individual, but in large populations with many loci each capable of more than one mutation then the problem of rarity is not so insuperable.

At a slightly sub-surface level, the author is directing attention to the beauty and intricacy of many natural processes. It is this aspect of the book which is most satisfactory, and it leaves the reader with a sense of marvel and maybe even of awe. The examples are chosen to fulfil this special purpose and they do so very well. His statement, however, that to scientists "the æsthetic appeal of their subject seems to be one that many of them feel ashamed of" is, I feel, unjust and very often untrue, for most scientists are conscious of the beauty that can and does lie in their material, in their apparatus, and in their experimental and mathematical proofs.

The third component of this book follows naturally from what I have called the sub-surface level, for he states that since the world of Nature is strange, beautiful, intricate, yet finely adjusted to operate as a unit, then there is "in it striking evidence of some directing intelligence" (p. 143). From this philosophic standpoint much of the material comes to be presented in a very teleological manner; for example, "marine animals . . . will avoid water that is fresh, because if they fail to do so they will perish" (p. 45). Such an attitude despises "the laws (if they can be called laws) of chance" (p. 124), and in consequence minimizes the achievements of modern genetics and replaces these by a mystic power. Thus evolution becomes a process in which "it is as though (and why not?) the change had been foreseen and allowed for in the course of the animal's evolution" (p. 140).

This is not a bad book. It is well written and illustrated, full of interesting examples and personal anecdotes, but the theme is too grand for 221 pages and its insistence on direction by a "guiding intelligence" may ring very strangely in the ears of those of its readers with a biological training.

Alan R. Gemmell

TREES AND SHRUBS

The Book of Flowering Trees and Shrubs

By Stanley B. Whitehead. Pp. 244+63 plates in colour. (London and New York: Frederick Warne and Co., Ltd., 1956.) 15*s*. net.

THIS pocket-size book is a companion volume to "The Book of Garden Plants" by G. A. R. Phillips, and is illustrated by the same artist, Miss Joan Lupton. It deals with shrubs, small trees and climbers, and by far the greater part of the text, 178 out of a total of 244 pages, is devoted to brief descriptions of 230 genera (not 240 as stated in the preface). Seven genera are divided into two or more parts, and each part is dealt with as though it was a genus, so that altogether there are 248 accounts of genera and subdivisions of genera, each with notes on the species or a selection of the species. The genera are arranged alphabetically, and each genus or subdivision is illustrated by a small coloured figure of one of its species. These figures, set four on a page, are sufficient to give the reader some idea of the sort of plant described, but they suffer the usual defect of their kind, namely, the almost complete failure to render the green of the leaves even approximately the right colour. The information given in the text is reasonably accurate, and the book may justly be regarded as "a simple and non-technical guide" to the choice, identification and qualities of flowering shrubs and small trees for the modern garden—which is what it is intended to be. As such, it should prove very useful. It must be pointed out, however, that many of the genera included are hardy only in the mildest parts of the British Isles.

In addition to the descriptive matter, there are chapters on shrubs in the modern garden, soils and situations, planting and propagating, and care of trees and shrubs. Short lists of species suitable for different soils and various situations are given, there are an illustrated glossary and, finally, an index. A reference to the method of propagation is given for each genus, and there are ten pages of details about the various methods; these details scarcely seem necessary in a book of this kind.

There are occasional examples of slipshod writing like "The genus is one of three species . . .", and the author has an irritating habit of using the word 'group' instead of 'genus'. The word 'trees' in the account of *Nuttallia* was no doubt a slip of the pen, and the same may account for the home of *Acer griseum* being given as Cochin China instead of Central China. *Stranvaesia davidiana* is a native of China, not of the Himalayas. *Rhododendron griersonianum*, shown on Plate 50 as an example of a hybrid rhododendron, is a good species, while *R. bullatum*, shown as a deciduous species, is an evergreen. One understands, and sympathizes about, the unfortunate oversight which led to the printing on p. 191 of "dry, splitting-capsule seed-vessel"—it happens to us all—but I have quite failed to elucidate "*M[alus] trilobata*, Syria, becomes a fine accent with columnar growth to thirty feet, . . .". Incidentally, this species is recorded from Palestine and the Balkan Peninsula in addition to Syria.

J. Robert Sealy

DYNAMIC BIOCHEMISTRY

Dynamic Aspects of Biochemistry

By Prof. Ernest Baldwin. Third edition. Pp. xx+526. (Cambridge: At the University Press, 1957.) 37*s*. 6*d*. net.

THIS has become a classic among biochemistry books. Prof. Baldwin received an international prize for writing it, and it has been translated into half a dozen languages including Russian and Japanese. It has long been a best-seller, valued alike by students and their teachers. It was natural, therefore, to feel anxiety on opening this new edition, lest the book might have lost its familiar and unique character. The first glance was reassuring. The author's well-known whirligig arrows, indicating coupled reactions, still cover the pages; one still learns that spiders excrete guanine; the sequence figs, fire-flies and fishes is still found in the new, shortened index. Having said this, the reviewer has to admit to a feeling of disappointment that some of the most outstanding advances of the past five years have not found their way into this new edition. Among these, the researches of Sanger on insulin and of du Vigneaud on the posterior pituitary hormones at once come to mind. Perhaps the author thought of these as structural rather than dynamic aspects, but he must have found it hard to exclude them. Again, some discussion of the biosynthesis of sterols and porphyrins and a more up-to-date account of the thyroid hormones would have been welcome.

The difficulty, of course, is to suggest what could have been left out to make room for new material, and opinions will, no doubt, differ on this. The book does not claim to be a complete text-book of biochemistry. Indeed, one of its great merits is that it is selective, and students will be thankful that its size (and its price) have not been much increased. There are, though, some sections, carried over from the original edition, which might perhaps have made way for more live topics. For example, less space might have been given to the descriptions and structures of some of the more uncommon nitrogenous bases. The obscure artefact creatone occupies half a page, though noradrenaline and 5-hydroxytryptamine are, apparently, not mentioned anywhere. The main consideration, though, is that Prof. Baldwin

has chosen to write on selected topics which interested him, and has done it supremely well, in language which is simple, forthright and vivid.

It would be wrong to give the impression that this edition does not contain any new material. To mention examples, there is a new chapter on the photosynthesis of carbohydrates in green plants, the chapters on the metabolism of carbohydrates and of fats have been substantially revised, the sections on purine synthesis, on oxidation systems and on subcellular fractions have been enlarged, and many small revisions have been made throughout the book. A good many misprints have been allowed to slip through, more than would have been expected in a book of this importance.

Biochemists will be grateful to Prof. Baldwin for rejuvenating his famous book and will wish it many further years of vitality. Of course it still carries its original dedication "To Hoppy". Gowland Hopkins's teaching and outlook still permeate it: there could be no higher recommendation. D. C. HARRISON

ALL ABOUT RHEOLOGY

Rheology

Theory and Applications, Vol. 2. Edited by Frederick R. Eirich. Pp. xiii+591. (New York: Academic Press, Inc.; London: Academic Books, Ltd., 1958.) 18 dollars.

IN his preface, the Editor discusses three criticisms from reviews of the first volume: "overlap or repetitions, unequal approach or even conflicting views, and non-uniformity of nomenclature" (see *Nature*, 179, 1207; 1957). If one is prepared to accept his views on these three points, there is indeed little to criticize in the second volume. Like the first, it contains a series of admirable essays on subjects of current rheological interest, by authoritative writers.

But I cannot altogether accept the Editor's dismissal of these earlier criticisms. While some overlapping is inevitable and even desirable, the size and the price of the book might have been reduced with some advantage by excluding material readily available elsewhere. One can scarcely suppose that this three-volume work, important as it is, will serve as the only source of information for any rheologist who is sufficiently advanced in his subject to follow its treatments. The most striking example is an otherwise excellent chapter on fibres by R. Meredith. This author edited and largely wrote a whole book[1] on this subject only two years ago. There would seem to be little advantage in including a fifty-page summary of this in the present work.

On the question of symbols, one appreciates the Editor's difficulties with the more specialized properties and concepts; but at least most English-speaking rheologists are already in fairly general agreement on the symbols which are most widely used. It is welcome news that "it is hoped that, by the time the third volume is ready to go to press, efforts to unify nomenclature may have reached a point where they can be applied to this book".

The subject-matter of the book divides itself naturally into three parts. The first seven chapters deal with mainly theoretical aspects of the rheology of high-polymers, Chapters 8–10 are concerned with gelatin, asphalts and geo-rheology, and the last three chapters describe experimental techniques. The middle section, especially the chapters on gelatin by A. G. Ward and P. R. Saunders and that on geological problems by B. Gutenberg, are particularly valuable, since the contents are not readily available elsewhere. The practical rheologist, for whom the earlier chapters are directly applicable to his everyday work, will get relaxation and intellectual pleasure from reading this most lucid account of how elastic moduli, viscosities and relaxation times are computed for the great masses of material which stretch for many miles beneath our feet.

Chapters 11 and 12, especially the latter, contain little that is not already on the shelves of most readers, but Chapter 13, on goniometric methods, by A. Jobling and J. E. Roberts, will suggest new and exciting possibilities to many practical rheologists.

This book, like its predecessor, is beautifully produced and printed; and Volume 2 is singularly free from misprints, though the printers still seem to have trouble (p. 207) with the spelling of "Treloar".

Such stringent criticism of a book is justified only if it is felt that the work is one of outstanding importance and of exceptional merit. In the present case there can be no doubts on this score.

G. W. SCOTT BLAIR

[1] Meredith, R., "Mechanical Properties of Textile Fibres" (North Holland Pub. Co., 1956).

WEIGHT REDUCTION IN THE HUMAN SUBJECT

This Slimming Business

By Prof. John Yudkin. Pp. 191. (London: MacGibbon and Kee, Ltd., 1958.) 15*s*. net.

THE medical advances, permitting more people of the Western World to reach old age, and the changed dietary habits of modern civilization associated with declining physical activity have focused attention on the need for weight control. Most accounts of this subject are either long scientific tomes on nutrition or uninformed accounts in the fashion or advertisement columns of newspapers and periodicals. This book claims to explain in non-technical language why some people are overweight and what they can and ought to do about it. It seems to be written mainly for women, but the information it presents could take an important place in the serious literature of the subject. It is, however, written in a chatty style more suited to the spoken than the written word and is likely to be appreciated more by those who would prefer to read a short newspaper article than the whole of this book.

The first seventy pages are concerned mainly with some general conceptions and misconceptions of obesity. An attempt to explain to the layman the significance of biological variation in relation to normal body-weight, although comprehensible to the scientific reader, seems to have been less successful with an overweight professional journalist of a national newspaper (*Daily Mail*, May 5, 1958).

There follows a brief but excellent account of nutrition that should enable the non-scientific reader to appreciate the reasons for the condemnation of much of the published nonsense on dieting. Pages 106–114 are not particularly illuminating—and with advantage could have been replaced by more compact pictorial diagrams.

The author then presents his case for controlling weight by reducing carbohydrate rather than fat

calories. One cannot accept his implied argument that modern man is healthier on the carnivorous diet than his primitive forbears, but the general idea is sound that the fundamental fault of the present-day diets is that increasing wealth permits increasing dietary protein and fat without corresponding decrease in the intake of foodstuffs such as cakes, ice cream, biscuits and sweets. There is no doubt that many people can lose excess weight with a diet low in carbohydrate but unrestricted in protein and fat. The case for not restricting protein is unanswerable; that for allowing unrestricted fat is on less certain ground. According to Prof. Yudkin, restriction of carbohydrate will usually be associated with relatively little, if any, increase in fat, but many people will take extra cream with their fruit and butter with their peas and beans. He wisely avoids discussing the possible effects of dairy fats and unsaturated vegetable fats in increasing or decreasing, respectively, the incidence of coronary disease.

In presenting his slimming regime, the author has taken a leaf from the notebook of the diabetic. Lawrence's line-ration scheme enabled the great majority of diabetics of widely differing mental acumen to control carbohydrate intake while retaining freedom of selection of foodstuffs. Lawrence's unit was the black portion corresponding to 10 gm. carbohydrate, and all the intakes of diet were tabulated according to the number of such portions they contained; Yudkin has selected 5 gm. carbohydrate as his unit, wisely since he hopes to restrict his slimming subject to 4 or less of such units, whereas diabetic patients require much more. This scheme provides an acceptable method of caloric reduction deserving wide use.

One can quarrel with little of the scientific content of this book, although the physiological reality of the 'appestat' is open to some doubt. Many authorities believe that water retention may play an even more important part than the author suggests in delaying weight reduction in subjects on diets of undoubted efficacy.

CHARLES H. GRAY

DYNAMICS OF FISH POPULATIONS

On the Dynamics of Exploited Fish Populations

By R. J. H. Beverton and S. J. Holt. (Ministry of Agriculture, Fisheries and Food—Fishery Investigations, Series 2, Vol. 19.) Pp. 533. (London: H.M. Stationery Office, 1957.) 126*s*. net.

THE cover jacket of this important work states that, "Many of Beverton and Holt's methods will be applicable to other living populations—animals or plants—and their theoretical framework should form part of the syllabus of teaching in agriculture, in ecology, in geography, in sociology, and in nature preservation". This is a wide claim but true enough in so far as the application of mathematics to the study of fish is ahead of its application to populations of land organisms. The book is in four parts, of which the first two are primarily theoretical representations of the four primary factors, recruitment, natural mortality, fishing mortality and growth. The application of the theoretical methods is developed in Part 3, concerned with the estimation of parameters, and in Part 4 where population models containing these parameters are used to investigate the reactions of fish populations to exploitation. The argument is here primarily concerned with North Sea plaice and haddock, but examples are taken from other fish, including perch in confined lakes, which offer particular opportunities for getting to grips with population problems.

Some readers, more interested in the practical applications than the theoretical analyses, will doubtless turn straight to Part 4, but this would do the book less than justice, because it is mainly in the mathematical treatment of the subject that new ground is broken. But, while the authors are to be admired for their enterprise after seven years of effort in compiling a work of such magnitude, bringing into prominence the role of mathematical models in ecological theory and application, their methods are not beyond criticism. The work would, indeed, have benefited by a closer revision, for some of the lucidity is lost at critical points. In the treatment of random migration, for example, the authors get into rather deep water because they do not distinguish clearly between the case where displacements (d) are constant in magnitude and that where the displacements are variable. In this way they confuse the mean value of d^2 with the square of the mean value of d, whereas in the analogy they draw with the kinetic theory, the former is twice the latter.

Proofs of essential theorems are usually given fully, but some readers may be discouraged by arrays of impressive symbols, contortions and rearrangements, where simple and lucid arguments are available. For example, pages 37 and 38 are devoted to demonstrating in a particular case that the total annual yield from a steady-state population is the same as the yield from a single recruitment class followed through its life, an obvious and general result which can be rigorously proved in a few lines without mathematics. Again, the theorem on pages 39–40 is a rather unnecessary exercise in elementary summation and integration, for it follows immediately from the more easily demonstrated theorem that an exponentially declining population grouped into year classes is geometric. There are several instances where the formulation of the problems could have been more fully stated with the exact conditions, and with their formal enunciation more prominently displayed and numbered. Some ambiguity might thereby have been avoided.

There is a useful and extensive bibliography which, however, appears somewhat deficient on certain central themes. No mention is made in the text of the classical work of Lotka on stable age-structures, and the growth equations discussed at length are attributed to Bertalanffy (1934) instead of Pütter (1920). A few references could well have been omitted, for example that to Pétart (1938), because the serious reader unacquainted with higher mathematics might misunderstand its humour.

These criticisms, however, do not detract materially from the high value of this book as a major contribution to fishery science and a source of reference and interest to workers in mathematical biology. He who thinks in terms of living organisms rather than of symbols will inevitably take some buffeting, but it is pleasing to find support given to "the tradition dating from T. H. Huxley's time, and reaffirmed by Graham, that the best management of fishing is to leave it alone until events show that intervention is necessary". There is a useful index.

E. B. WORTHINGTON
J. G. SKELLAM

BIOCHEMISTRY OF CELLULOSE AND RELATED POLYSACCHARIDES*

By Dr. B. A. STONE
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THE naturally occurring β-glucans (polymers of β-D-glucopyranose) which are listed in Table 1 form a series of structurally related polysaccharides. Many of these glucans, for example cellulose, callose and yeast glucan, are constituents of cell walls; while others, such as the *Pneumococcus* type III and *Rhizobium* polysaccharides, occur as capsules associated with cell walls. A number of them are intracellular, for example laminarin and lichenin, and may be regarded as reserve polysaccharides, while others are extracellular and of unknown significance (the β-glucans of bacteria and fungi).

Cellulose is the most abundant β-glucan; its structure and organization in the cell walls of higher plants have recently been reviewed[1]. The β-1 : 3 glucans are less well known, although as early as 1890 Mangin[2] reported the occurrence of an insoluble polysaccharide in the sieve plates of the phloem and in pollen tubes and pollen grains. The polysaccharide, which he named callose, he distinguished from cellulose by its insolubility in cuprammonium and by differential staining reactions. Callose replaced cellulose, or occurred with it, in the mycelium and fruiting bodies of many families of fungi, yeasts, lichens and some algæ. These observations have not been confirmed in any detail, but three β-1 : 3 glucans, callose, pachyman, and yeast glucan have similar properties to Mangin's callose and are found in similar situations.

Several β-glucans with both 1 : 4 and 1 : 3 linkages are found naturally; those which occur in cereal grains are important in the germination process[3]. Thus oat glucan is located on the surface of the kernel, and during germination, it has been suggested, it is dissolved away by β-glucanases allowing access into the endosperm of enzymes concerned with the mobilization of starch[4].

Biosynthesis

Our knowledge of the biosynthesis of β-glucans has, until recently, been limited, and most of our information relates to cellulose synthesis. Analytical studies of the changes in carbohydrates in the developing cotton plant[5] and of the pathways of carbohydrate metabolism in *Acetobacter xylinum*[6] have led to the suggestion that hexose phosphate is involved in cellulose synthesis. In neither system was cellobiose utilized; thus, although transglucosylations involving the formation of oligosaccharides from cellobiose have been reported, they do not appear to be concerned in cellulose synthesis[7]. Experiments involving the incorporation of labelled carbon, administered as monosaccharides, into the cellulose of *Acetobacter* spp.[8] and of cotton[9] and wheat plants[10] have shown that the C-1 of glucose-1-^{14}C is usually retained to the extent of 60–80 per cent in its original position, although the conditions of the experiment and the physiological status of the plants greatly influence the amount of retention. These results suggest that,

Table 1. Basic Structures and Distribution of β-Glucans

	Ref.	Main linkages	Other linkages	Other monomer units	Distribution
Cellulose		1–4	—	—	Cell walls throughout plant kingdom Test of Tunicates, mammalian tissues ?
Pachyman	*a*	1–3	—	—	*Poria cocus* sclerotia
Yeast glucan	*b*	1–3	1–2	—	Cell walls of some yeasts, for example *S. cerevisiae*
Callose	*c*	1–3	—	Uronic acid (2 per cent)	Sieve tubes in phloem of grape vine
Laminarin	*d*	1–3	1–6	Mannitol (2–3 per cent)	Fronds of some Phaeophyceae
Lichenan	*e, f*	1–3 (30 per cent) 1–4 (70 per cent)	—	—	Fronds of some lichens, notably *Cetraria icelandica*
Oat glucan	*g*	1–3 (30 per cent) 1–4 (70 per cent)	—	—	Oat grain
Barley glucan	*h*	1–3 (50 per cent) 1–4 (50 per cent)	—	—	Barley grain
Pneumococcus type III polysaccharide	*j*	1–3 (50 per cent) 1–4 (50 per cent)	—	Glucuronic acid (50 per cent)	*Diplococcus pneumoniae* type III capsule
Rhizobium polysaccharide	*k*	1–4	1–6	Glucuronic acid	*Rhizobium radicicola* capsule
Pustulan	*l*	1–6	—	—	Fronds of the lichen *Umbilicaria pustulata*
Luteic acid	*m*	1–6	1–3, 1–4	Esterified malonic acid	Culture media *Penicillium luteum*
Crown gall polysaccharide	*n*	1–2	?	—	Culture media *Phytomonas tumefaciens*

(*a*) Warsi, S. A., and Whelan, W. J., *Chem. and Indust.*, 1573 (1957); (*b*) Bell, D. J., and Northcote, D. H., *J. Chem. Soc.*, 1944 (1950); (*c*) Aspinall, G. O., and Kessler, G., *Chem. and Indust.*, 1296 (1957); (*d*) Peat, S., Whelan, W. J., and Lawley, H. G., *J. Chem. Soc.*, 724, 729 (1957); (*e*) Chanda, N. B., Hirst, E. L., and Manners, D. J., *J. Chem. Soc.*, 1951 (1957); (*f*) Peat, S., Whelan, W. J., and Roberts, J. G., *J. Chem. Soc.*, 3916 (1958); (*g*) Acker, L., Diemar, W., and Samhammer, E., *Z. Lebensmitt Untersuch.*, **102**, 225 (1955); (*h*) Aspinall, G. O., and Telfer, R. G. J., *J. Chem. Soc.*, 3519 (1954); (*j*) Reeves, R. E., and Goebel, W. F., *J. Biol. Chem.*, **139**, 511 (1941); (*k*) Schlüchterer, E., and Stacey, M., *J. Chem. Soc.*, 776 (1945); (*l*) Lindberg, B., and McPherson, J., *Acta Chem. Scand.*, **8**, 985 (1954); (*m*) Lloyd, P. F., Pon, G., and Stacey, M., *Chem. and Indust.*, 172 (1956); (*n*) Putnam, E. W., Potter, A. L., Hodgson, R., and Hassid, W. Z., *J. Amer. Chem. Soc.*, **72**, 5024 (1950).

* Based on two lectures delivered at University College, London, on May 5 and 12.

although some glucose can be polymerized directly into the cellulose molecule, a proportion of the monomer units of the cellulose arise by resynthesis of fragmented glucose molecules. Similar experiments have been extended to the xylan of the wheat plant by Altermatt and Neish[10]. The results suggest that the xylose units arise by the oxidation of the hexose at C-6, followed by decarboxylation.

In cell-free systems from *A. xylinum*, prepared by Mickle disintegration[11] or glass-grinding of freeze-dried cells[12], cellulose synthesis can occur when glucose and adenosine triphosphate are added. Greathouse[12] showed that 96·7 per cent of the label was retained in the C-1 of the glucose units when glucose-1-^{14}C was used as substrate.

Uridine diphosphate-glucuronic acid and uridine diphosphate-glucose have been established as glycosyl donors in the formation of glucuronides[13] and disaccharide phosphates[14] respectively. Altermatt and Neish[10] have suggested a scheme for the synthesis of plant polysaccharides in which these types of compounds are envisaged as donors, or close precursors of donors, of the polymerized units. Their ideas are supported by the isolation, from mung bean seedlings, of a number of nucleotide glycosyl compounds and of enzyme systems involved in their formation and interconversion[15]. They are further supported by a report[16] of the synthesis of cellulose by cell-free preparations of *A. xylinum* from the labelled glucose of uridine diphosphate-glucose. Uridine nucleotide glycosyl compounds have also been implicated in the synthesis of chitin, hyaluronic acid and glycogen[17], on the basis solely of experimental evidence of the incorporation into the polysaccharide of labelled monomer supplied as the nucleotide glycosyl compound. Whether the monosaccharide of the nucleotide sugar derivative is transferred directly by a single enzymic step or whether indirectly by several steps is unknown. Until the synthesizing systems have been further examined and purified the exact role of the nucleotide derivatives will remain uncertain.

Among the β-glucans there exist representatives of many of the types of structures encountered among the polysaccharides, including unbranched chains, involving the same positional linkage type between each unit as in cellulose or two different linkage types as in lichenan, and branched chains. Among related polymers are structural types in which different monomer units are joined by linkages of the same positional but different configurational types, for example the pneumococcal type VIII capsular polysaccharide which has the repeating unit[18]:

$$\text{-}\beta\text{-D-}Gp\text{A-}(1\rightarrow 4)\text{-}\beta\text{-D-}Gp\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-}Gp\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-}Gal p\text{-}(1\rightarrow 4)\text{-}$$

(where GpA = glucopyranuronic acid; Gp = glucopyranose; $Gal p$ = galactopyranose), and a further type, often found among the xylans, has short side chains of xylose or other sugars attached to the main β-xylose chain.

This wide range of structural types makes any generalized statement about the mechanism of synthesis difficult, but clearly two systems would be required, the first concerned with the provision of building units of the right configuration and state of activation, which could be utilized by the second or polymerizing system and built into the characteristic structure of the polymer. We know that both these systems are under genetic control[19], and although there is some information concerning the provision of donor molecules the polymerization system for β-glucans is not understood at all. Two possibilities exist for this system. First the synthesis may be directly under enzymic control as it is, for example, in the synthesis of glycogen. However, for the synthesis of polysaccharides having different linkage types and perhaps different monomer units, the number of enzymes required would be large, their specificities restricted and often novel in character in order to direct the synthesis in a reproducible manner. The second possibility, that the synthesis is under the control of a template, has been put forward, and is considered in detail by Wilkinson in his recent review on bacterial polysaccharides[19].

It is possible to envisage the synthesis of certain polysaccharides, for example lichenan, pneumococcal type III and VIII polysaccharides (Table 1), as a polymerization of a preformed repeating unit by transglycosylation from a suitable donor. Here the sequence of units in the repeating block and their mode of linkage would be controlled either by a number of specific enzymes or by a template mechanism. Some support for this type of process is provided by the evidence, summarized by Park[20], that the nucleotide sugar amino-acid compounds, which he isolated from cultures of penicillin-inhibited *Staphylococcus aureus*, are involved in the synthesis of the bacterial cell wall.

Several of the β-glucans, including bacterial cellulose, occur extracellularly. The problem of their synthesis and appearance outside the cell is analogous to that posed by the occurrence of other high molecular weight compounds in the medium surrounding cells, for example exo-enzymes, hormones and antibodies. This problem is related in a direct way to the mechanism of the synthesis. For certain types of polysaccharides such as dextrans and levans, the synthesis can occur in the extracellular medium if suitable donor molecules are provided. For extracellular protein and probably for many polysaccharides the building units are only found intracellularly together with the system which controls the specific arrangement of the units in the polymer. This raises the problem of the selective release from the cell of the macromolecule. In the case of the bacterial cell wall complex and the capsular and extracellular polysaccharides it is possible to envisage an enzymically controlled synthesis at the cell surface (plasma membrane) or the formation there of activated units which can be polymerized extracellularly by extracellular enzymes. The problem is well illustrated by the synthesis of bacterial cellulose, where electron microscope studies indicate that the cellulose microfibrils are formed in the medium remote from the cells[21]. If this is correct then the immediate donor of the polymerized units may be found in the medium together with the transferring enzyme. Although uridine diphosphate-glucose is implicated in cellulose synthesis[16] it is unlikely to be an extracellular donor since the nucleotide portion would be lost to the cell. Some intermediate reactions appear likely. A speculative scheme based on an analogy with the formation of lactose, trehalose and sucrose phosphates[13] from uridine diphosphate-glucose and glucose-1-phosphate,-6-phosphate, and fructose-6- or 1-phosphate might involve the formation of cellobiose phosphate as an intermediate, perhaps at the plasma membrane surface. Transfer of cellobiose from its phosphate to suitable acceptors (primers) would result in the formation of cellulose molecules.

Cellobiose has something to recommend it here since it is the true repeating unit of cellulose. The isolation of an oligosaccharide phosphate and cellodextrins from the medium of actively synthesizing *A. acetigenum* cultures is consistent with this scheme[22].

Depolymerization

The enzymic depolymerization of cellulose and other β-glucans has not been investigated at the biochemical level with the same intensity as has starch degradation. For cellulose the heterogeneous nature of the reaction system and the complexities raised by the supramolecular structure of the substrate have been the chief check to progress.

The facility with which bacteria and fungi attack cellulose contrasts with the slow and incomplete attack by cell-free enzyme preparations on organized celluloses. The difference has been argued as being due to the loss of labile enzymes in preparation[23], although the characteristic localization of the attack by bacteria and fungi suggests that the enzyme concentration at the site of dissolution, the localization of the cellulase at the cell surface in bacteria[23], and removal of reaction products might also be important factors in explaining the difference.

The effect of the supramolecular structure of the substrate on the action of cellulases has been thoroughly investigated[24], and it is clear that the attack is initially limited to the regions of low lateral association of the cellulose molecules and that any treatment of the cellulose which will increase the proportion of the less ordered regions will increase the extent of hydrolysis. Frequently the attack on the molecular chains has been shown to be by a random mechanism[25], and although there are reports that endwise attack on the chains can occur the evidence is indirect[3, 26].

Whitaker[27] has shown that a fractionated and apparently unienzymatic cellulase preparation from the mould *Myrothecium verrucaria* can hydrolyse cellulose-type molecules with lengths corresponding to a degree of polymerization of 3 and upwards. Thus a single enzyme may be responsible for an almost complete depolymerization, although the results of analysis of crude cellulase preparations from *M. verrucaria* and other fungi by a variety of fractionation procedures suggests that they are complex systems with several active components[28]. Unfortunately the components have not been obtained in a state which would allow differences in their chemistry and specificity to be clearly demonstrated.

Many cellulase preparations also contain a cellobiase and it was early suggested that cellulose hydrolysis might be a two-step process :

$$\text{cellulose} \xrightarrow{\text{cellulase}} \text{cellobiose} \xrightarrow{\text{cellobiase}} \text{glucose}$$

In some cases cellobiase activity is not limited to the disaccharide as is shown by the properties of the two fractions obtained by alumina chromatography of *Aspergillus oryzae* preparations[29]. One was termed an 'oligase' since it hydrolysed cellodextrins with degrees of polymerization 2–6 but longer chains weakly or not at all. The other termed a 'polyase' hydrolysed cellodextrins and cellulose. The activities of the two components overlapped at the cellohexaose level. A similar separation has been achieved in *Aspergillus niger* preparations by gradient elution from anion exchange columns[30]. The oligase components appear to be similar to the cellobiase reported recently to occur intracellularly in *Stachybotrys atra* mycelium and to hydrolyse cellodextrins up to degree of polymerization 11, but not sodium carboxymethylcellulose or alkali swollen cellulose[31]. A polyase component with chain length specificity resembling Whitaker's *Myrothecium* preparation has already been studied in *S. atra*[32]. The scheme of cellulose depolymerization in these fungi might then be :

$$\text{cellulose} \xrightarrow{\text{polyase}} \text{cellodextrins} \xrightarrow{\text{oligase}} \text{cellobiose}$$

The synergistic action between the two components would lead to a very rapid depolymerization.

The turnover number of Whitaker's *Myrothecium* enzyme[27] reached a maximum value at degree of polymerization 4, and below this value the activity dropped off rapidly, suggesting that the upper limit for the combining site of substrate with enzyme was about four glucose units long, which closely resembles observations made with respect to the combing site of iso-maltodextrins for antibodies against dextrans[33]. *S. atra* cellobiase splits off glucose units from the end of the dextrin[34], and its activity is independent of chain length over the range of cellodextrins tested (up to degree of polymerization 11). The combining site here may only involve one cellobiose unit at the chain end, so that the length of the chain would not influence the activity until it became long enough to create difficulties in the orientation of the chain end with respect to the active site.

The depolymerization of β-1 : 3 glucans requires specific enzymes and it has been possible to separate β-1 : 3-ase activity from the poly-β-1 : 4-ase activity in *A. niger* preparations either by chromatography on calcium phosphate or anion exchange resin columns[30,35]. This linkage specificity was considered by Ploetz and Pogacar[36] in explaining kinetic data obtained with *Helix pomatia* preparations; they, however, favoured an interpretation based on the presence of a non-specific oligase and polyase which could hydrolyse β-1 : 4, 1 : 3 and 1 : 6 linkages.

Following the elucidation of the structure of lichenan we are in a position to re-evaluate some of the findings of earlier workers who regarded lichenan as another form of cellulose. It is possible that both 1 : 4 and 1 : 3 hydrolysing enzymes would degrade this polymer provided that the presence of adjacent linkages of the other type do not restrict the hydrolysis. The 'lichenase' activity of *A. oryzae* can be fractionated[37] by precipitation with ether-alcohol mixtures into a fraction with activity on lichenan but not on cellulose and two fractions with activity towards both substrates. The activity-*p*H curves for the two substrates are very different and show multiple optima. These observations can be interpreted in terms of separate enzymes, one specific for 1 : 4 linkages and the other specific for 1 : 3 linkages. However, recent observations using the separated components of the *A. niger* preparation[30] have shown that only the poly-1 : 4-ase and the β-glucosidase components hydrolyse lichenan. The presence of 1 : 4 linkages on either side of the 1 : 3 linkages apparently prevents their hydrolysis by the 1 : 3-ase.

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VISUAL PIGMENTS IN THE COLOUR BLIND

By Dr. W. A. H. RUSHTON, F.R.S.

NEARLY all colour-blind subjects confuse red and green, and the most extreme cases are dichromats, which means that they can match every colour of the spectrum with a mixture, say, of red and blue. But these dichromats are of two sharply different kinds, for the 'protanope' is insensitive to light of long wave-length and needs a very bright red light to match a moderate green, whereas the 'deuteranope' is nearly normal in his appreciation of relative brightness throughout the spectrum. Now when we plot upon the colour triangle those hues which the dichromats confuse, the points fall upon concurrent straight lines. For example, the protanope confusion loci radiate from the red corner of the colour triangle and, as Helmholtz and Maxwell pointed out long ago, this supports the view that the red 'mechanism' is absent. For this reason, protanopes are often called 'red blind'. The deuteranope lines are more nearly parallel to the red–green side of the triangle, so the name 'green blind' is less obviously applicable, and Pitt[1] has favoured the possibility suggested by Helmholtz that no sensation is lost but that red and green are fused. I have always had difficulty in grasping clearly what was the kind of thing which might in the deuteranope either be lost or fused. But now I wish to propose the view that there is both loss and fusion. What is lost is one class of cone, namely, that which in the normal is 'green-sensitive'; what is fused is the visual pigment, for the only non-blue cone which the deuteranope possesses contains the red- and green-sensitive pigments mixed together.

The pigments in the cones may be measured upon the fovea (which is rod-free) by the method of reflexion densitometry (Rushton[2]). When this is done in the red–green region of the spectrum with a protanope subject, one pigment is found. Fig. 1 shows its difference spectrum (the change in absorption after photolysis produced by shining a very bright light into the eye). The difference spectrum is the same with partial photolysis from red or from green light; hence only one visual pigment is present. If this is the pigment serving protanope vision, the curve might be expected to correspond to his daylight spectral visibility, just as twilight visibility corresponds to the absorption spectrum of rhodopsin (= visual purple).

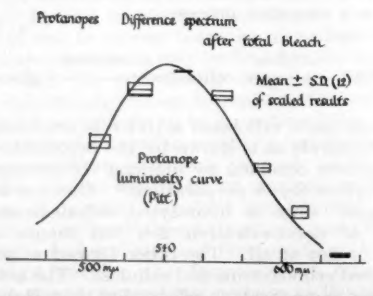

Fig. 1. Difference spectrum in the protanope, that is, the change in the logarithm of the reflectivity of the eye for light of various wave-lengths when the visual pigment has been bleached away by strong light

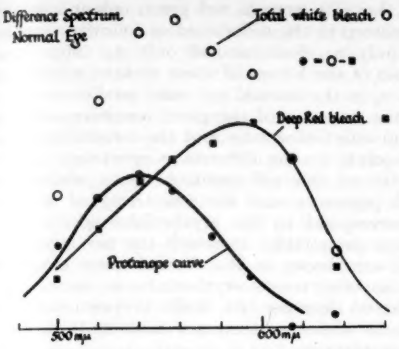

Fig. 2. Pigments in the normal eye analysed by bleaching with coloured lights. The total pigment is made up of chlorolabe and erythrolabe in the proportions shown. There is about twice as much chlorolabe in the protanope as in the normal

The curve of Fig. 1 is, in fact, Pitt's[1] protanope visibility, so it seems clear that protanopes can see only one colour in this half of the spectrum because they possess only this one visual pigment with absorption there. In contrast, Fig. 2 shows the results of partial photolysis of the normal fovea using red and green lights. This reveals two distinct pigments. One is the pigment of the protanope but in rather small amount; the other is a pigment which absorbs maximally at about 590 mμ and predominates. This latter pigment may be named 'erythrolabe' (= red taking), the protanope pigment 'chlorolabe', and the blue-sensitive pigment which does not here concern us, 'cyanolabe'. The protanope is known to have the normal number of cones in his fovea and normal acuity in his vision. Clearly, his loss is that none of his cones contains erythrolabe, but all are filled instead with chlorolabe.

A symmetrical situation would be to suppose that the cones of the deuteranope contained nothing but erythrolabe, but that is not true. Not only is the deuteranope visibility curve Fig. 4 (from Pitt[1]) very different in shape from the difference spectrum of erythrolabe, but also partial photolysis with a red or green light reveals (Fig. 3) that both erythrolabe and chlorolabe are present. Thus, the deuteranope visibility is monochromatic in the red–green, not because he lacks either of the normal pigments, but

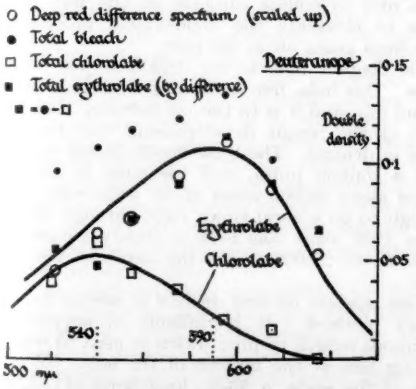

Fig. 3. Pigments in the deuteranope in correct proportions. Erythrolabe estimated by difference in total bleach (squares) and directly by bleaching with deep red light (circles)

he mixes the information which separately they could give. This might be because the pigments are mixed in a single class of cone, or because the cone signals are mixed in a single class of nerve. The following two experiments point rather to the former explanation.

A 4° circular field was made up of seven parallel strips of alternate green and magenta light, the blue and red of the magenta being separately adjusted by the deuteranope so that all seven strips appeared identical to him. Now a deep red field was superposed uniformly over the whole 4° of matching strips, and increased in intensity up to the point where the 4° circle was no longer visible. At no stage did the green appear different from the magenta; nor did any difference appear when a bright green field instead of a red one was uniformly superposed.

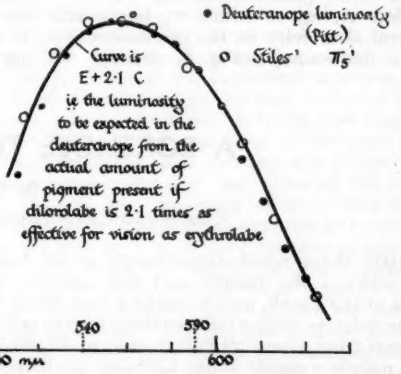
Deuteranope luminosity (Pitt)
Stiles' π_5'
Curve is E + 2·1 C is the luminosity to be expected in the deuteranope from the actual amount of pigment present if chlorolabe is 2·1 times as effective for vision as erythrolabe
540
590
500 mμ
600

Fig. 4. Deuteranope luminosity curve (dots) from Pitt. Stiles's red sensitive mechanism π_5' (circles). The curve is the expectation from the pigments found in the deuteranope if chlorolabe is 2·1 times as effective for vision as erythrolabe. π_5' is the high-intensity form of π_5 (see Stiles, ref. 4)

When I myself looked at the circle through the strong green superposition, the green stripes were scarcely noticeable, but the magenta stripes stood out brightly. The reverse was the case when red light was superposed, and it was hard to believe that the deuteranope could find the two sets of strips equal. But his repeated settings of the red intensity for a complete match showed a divergence which was certainly less than 5 per cent when red, green or no fields were superposed.

In the second experiment, the 4° circle was half green and half magenta of the shade which exactly matched it for the deuteranope. A small point of light, either red or green, was flashed upon either one half of the field or the other, the flash being 10 msec. in duration and repeated once per sec. If the threshold for a green flash upon a red field is denoted (G/R), it was found that the ratios $(G/G)/(G/R)$ and $(R/R)/(R/G)$ were each nearly unity, and their product was exactly unity. For my (normal) eye it was about 8.

These two experiments prove that whatever is mixed in the deuteranope is mixed peripheral to that place in the retina where light adaptation occurs, and before the site where the cone signal becomes a somewhat logarithmic function of the light intensity. It appears, therefore, to arise peripheral to the place where the electroretinogram is generated, which Brindley[3] locates at the rods and cones. So, if we

accept this, the deuteranope has his pigments mixed in a single class of cone. We cannot say in advance what is the relative efficacy of erythrolabe and chlorolabe in producing a nerve signal, but we may deduce it by noting how greatly the chlorolabe curve of Fig. 3 must be scaled up so that when added to the erythrolabe curve it will correspond to the deuteranope visibility curve; the factor is about 2. The curve of Fig. 4 gives the combined effect of erythrolabe and chlorolabe so estimated, and is seen to correspond well with Pitt's visibility curve (dots).

In work extending over twenty years, Stiles[4] has investigated colour mechanisms in normal subjects by measuring the increment thresholds of flashes of one colour upon the background of another colour—a method crudely followed in my deuteranope experiment described above.

In the red–green region of the spectrum, Stiles has found two quasi-independent colour mechanisms labelled π_4, π_5, of which π_4 has nearly the same spectral sensitivity as the protanope (Fig. 1) and π_5 as the deuteranope (Fig. 4, circles). We may say, then, that the normal red–green colour mechanisms are reduced in the dichromat as follows: protanopes have only π_4, deuteranopes only π_5. But from our analysis of the foveas of these dichromats the nature of π_4, π_5 in the normal eye may now be stated. For π_4 is the response of the green-sensitive cones which contain only chlorolabe and the sensitivity of which corresponds to the chlorolabe spectrum. π_5 is the response of the red-sensitive cones which contain mixed pigments and the sensitivity of which does not correspond to the erythrolabe spectrum. The physical proportions in which the two pigments are mixed are shown in Fig. 3, but since chlorolabe is twice as effective as erythrolabe in exciting vision, we should increase its scale proportionally in all problems where we turn our analysis from pigments to sensations.

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A BOREHOLE TO THE EARTH'S MANTLE?

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THE Mohorovičić discontinuity is the boundary between the mantle and the assorted surface rocks of the Earth, and it marks a very sharp change in the velocity with which earthquake waves travel—a jump from about 21,000 ft./sec. to 27,000 ft./sec. The mantle extends about half-way to the centre of the Earth, and encloses a core which earthquake seismic waves demonstrate to be fluid. At last year's meeting of the International Union of Geodesy and Geophysics at Toronto a resolution was passed urging the importance of drilling a bore-hole deep enough to penetrate the rock layer that lies below the Mohorovičić discontinuity.

The use of artificial earthquakes has been common practice for many years now in studying the Earth's crust. The seismic prospecting methods used to locate underground oil reservoirs tell the approximate depth and the shape of the surface of the underground layers of rock, but they do not give a firm indication of the type of rock. In order to draw an unambiguous picture of the sub-surface geology, it is necessary to follow particular rock layers from their surface outcrops, or from bore-holes. The layer below the Mohorovičić discontinuity does not come to the surface and therefore it is necessary to drill in order to find out what is the material—what chemical or radioactive properties it has—that forms the Earth's mantle.

It is found that the mantle lies at a depth of about 20 miles under the land, but that underneath the deep oceans it is only about 7 miles below sea-surface. The nearest the mantle comes to the surface of land is under oceanic islands, and here it is depressed below normal oceanic level to about 10 miles below surface. This is probably the best place to drill the hole to the Mohorovičić discontinuity.

The seismic measurements in the oceans enable estimates to be made of the thickness of sediment that has collected and compacted over millions of years on the sea-bed. As with oil-finding seismic work on land, the support of an outcrop or a bore-hole is needed to make an unambiguous interpretation of the measurements. In particular, there is a hard layer, lying beneath about 1,500 ft. of soft sediment, which could be a band of cemented limestone, or a continuous layer of volcanic rock. Beneath this again is a layer of rock which is probably the primeval ocean floor. The seismic measurements combined with analyses of volcanic material erupted from inside the Earth give some idea of what this rock might be, but they are only guesses because the exact origin of the molten lava of the volcano is not known, and in any event mixing will occur as the lava is forced upwards. A second recommendation was therefore made at Toronto—to drill a few thousand feet through the sediments that cover the three-mile deep oceans to see both what kind of rock lies beneath them and to collect samples all the way down in order to elucidate the depositional processes that have been going on in the past.

The deepest hole so far drilled is just over four miles. This hole, like most deep borings, was drilled to find oil—and it is to the oil industry that we owe most of the recent developments that have taken place in drilling. The total depth drilled is now well over a million miles, and last year in the United States alone 40,000 miles of oil wells were drilled—enough to go several times right through the Earth. Since 1927 there has been a steady increase, from 8,000 ft. to 22,000 ft., in the depth of the deepest hole.

Most modern oil-well drilling is carried out by the rotary method. It is difficult to appreciate the enormous length of pipe which is used to rotate the cutting tool at the bottom of the hole. To give an idea of the scale, a 33-ft. long wire, of 7/1,000 in. diameter, has about the same length-to-diameter ratio as a 20,000 ft. length of 4-in. drill-pipe. The

* Substance of a Royal Institution Discourse entitled "Drilling Deep Holes in the Earth", delivered on June 11.

cutting tool cannot be forced into the rock by pressing on such a long flexible length of pipe because the pipe would buckle. The tool is therefore driven into the rock by about 20–30 tons of extra thick pipe at the bottom of the hole, and the main length of the drill-pipe supports and rotates this bottom section. Without the heavy rigid section the drill-pipe bends, and the friction between the drill-pipe and the bore-hole almost stops the rotation; furthermore, the bit tends to go crooked. With a heavy section at the bottom, the thin drill-pipe is held in tension and the rock is penetrated quite readily. The motive power for the bit would be better at the bottom of the hole, and electric motors have been tried but without great success. It is difficult and perhaps dangerous to handle the large electric power needed, with cables that are subjected to the wear and tear of running in and out of a bore-hole.

Much more commonly used is a multi-stage turbine motor, which is driven by mud and water pumped down the drill-pipe and up the space between the drill-pipe and the walls of the hole. The driving motor consists of a hundred rotor elements, which give enough power to rotate the bit many times faster than the conventional rotary systems. The friction of the drill-pipe all the way down the walls of the bore-hole limits the rotary drilling speed to about 120 rev./min. High speed of rotation means correspondingly faster rock penetration; but apart from this, light-weight drill-pipe can be used, since it only carries the weight of the turbine, and does not have to twist it around; and, since the pipe is not rotated, there is very little wear on the sides of the bore-hole. Furthermore, if the cutting bit jams on an extra tough piece of rock, the turbine will be halted but the mud will circulate and keep the hole free. With the rotary drill, jamming of the bit often means that the drill-pipe is twisted until it breaks off.

The turbo-drill has another potential advantage which has not yet been realized. Because the drill-pipe is not rotated, the turbo-drill may be made to alter course by setting the axis of the drill at a few degrees to the drill-pipe with an elbow joint. There is no reason why this process could not be in some way controlled from the surface, so that the drill could be virtually steered in any direction. This is a desirable feature, because sometimes it is necessary to drill holes that are deviated sideways; for example, when producing oil from strata below a town where drilling would be inconvenient. However, it may also be the means of speeding up rate of drilling. The rate of penetration of the drill is approximately proportional to the weight which is allowed to press on the bit at the bottom of the hole; but there is a limit to the weight because in a narrow bore-hole the weight must be distributed over a length of heavy drill-pipe. Any slight bending of the pipe causes the drill to go off course, and this effect is often accentuated by the non-uniformity of the rock strata. If the drill can be steered along the true course, then we can press harder on the drill bit while still keeping straight and thus penetrate the rock correspondingly faster.

Even if we could drill very fast indeed, we should still have to spend time every now and again pulling up all the drill-pipe to renew the drilling bit when the cutting teeth became worn, or when the roller bearings which support the toothed wheels seize up. The time taken to unscrew and stack a few miles of drill-pipe is several hours. Much engineering development has gone into improving the life of drilling bits, and a recent advance has been to construct a bit which can be folded up and pulled up the inside of the pipe on a wire, so that a replacement can be lowered to carry on drilling without any need of removing the pipe itself from the hole. It is obviously much faster to wind up a wire on a drum than to unscrew all the pipe. This device is in its infancy, and a more revolutionary approach is to speed up the actual pulling out and unscrewing of the drill-pipe by means of a double hydraulic lift. The first lift raises a length of pipe, then the second lift raises the next section while the joint is being unscrewed and the first length of pipe is being stacked away. The first lift now goes down to catch hold of a third length of pipe and so a continuous hand-over-hand process goes on. These improvements in complete automation of pipe handling will not only save time and labour on the rig, but they will also give more carefully controlled operation. Automatic drillers are in fact coming into common use, and the servo system will be even better when used with smooth hydraulic feed instead of lifting by cable. The driller's job is not made less important, however; in fact, it is becoming more skilled and technical with each new advance. One of the problems that requires expert knowledge is the behaviour of the mud that is circulated down the drill-pipe and which flows up the space between the pipe and walls of the hole, carrying with it the fragments of rock that have been cut by the bit. These chippings must be removed, and only recently have laboratory experiments shown that very rapid removal of the chippings is important. Almost as much work must be done to grind up a chip as is required to form the chip from the parent rock in the first place. For efficient drilling, then, a chip must be removed before the next cutting tooth of the bit reaches it. Special outlets are made in modern drill bits to direct a stream of mud in the right places to do this. In order to carry the rock chippings to the surface, the mud must move at a few feet per second, and in order to minimize the pump power required the viscosity of the mud must be kept down.

However, the mud has other jobs to perform, and the final mixture is usually a compromise. In the first place, the mud must not settle out into its constituent parts if left standing, because if it did, the solid material sinking to the bottom would jam the drilling tools in the ground. A mixture of ground-up shale and water separates out into its constituent parts, but the addition of a small proportion of bentonite clay maintains the shale particles in suspension and so turns the mixture into a drilling mud.

Ideally, a thin layer of dried mud cake forms over the walls of the bore-hole, and in this way preserves the mud from contamination with any soluble compounds in the rock that is being drilled. At the same time, the mud cake on the walls of the hole gives strength to resist crumbling of soft materials such as clays and shales. The weight of the mud column itself holds back the walls of the hole from caving, in much the same way as pit-props support the roofs of coal mines. A good mud has almost jelly-like properties to stop it settling and to hold up the rock chippings when drilling stops. However, it must also be easy to pump down the drill-pipe—and so must be thixotropic.

Not only does mud help to keep the walls of the bore-hole from collapsing, but it also holds back high-pressure fluids—which may be water, oil or

gas—from entering the bore and causing trouble at the surface. It is unlikely that high-pressure gas will be encountered in drilling the 10-mile deep hole to the Mohorovičić discontinuity. Borings at Bikini and Eniwetok have already demonstrated that hard basaltic rock is reached at a depth of a few thousand feet, and seismic measurements have suggested that this basaltic rock continues uniformly to the 10-mile depth of the Earth's mantle. This tough basaltic rock will not need a protecting wall of mud because it is rigid enough to stand up securely on its own. The top part of the hole, which will pass through possible loose and porous coral rock, can be walled off with a strong steel casing. The main part of the hole will, however, present new problems, and in some ways these may be more akin to those met in gold prospecting than in looking for oil. Oil is not found in hard igneous rocks, and normally these are not encountered in oil-well drilling. On the other hand, bore-holes up to 6,000 ft. in length have been drilled in order to assess the potential of gold-bearing quartz strata. It is possible that water will suffice instead of mud as our circulating fluid. Some form of circulating fluid will almost certainly be needed, because although the rock may stand up without the assistance of a hydrostatic column, some cooling and lubricating of the drill bit will be required. The temperature ten miles deep is expected to be anywhere between 400° and 700° F. The pressure of the water column will prohibit boiling down the hole, but it will be necessary to cool the water when it reaches the surface. There will be no fundamental difficulty in this; in fact, it might be possible to circulate sea-water. The most likely site for the bore-hole in order to encounter the Mohorovičić discontinuity at as shallow a depth as possible will be on the edge of a coral atoll, so that sea-water will be one commodity in ample supply.

Rock-drilling in mining is often carried out using diamond bits, and the hard basalt that will be met in a deep bore-hole will probably best be cut in this way. With the high-speed rotation of the turbo-drill it might be possible to increase the normal rate of a few feet an hour to, say, ten feet an hour. At this rate it would take more than two hundred days drilling to penetrate 50,000 ft. of rock. The time taken to pull out and renew the drilling bit may be greater than this. At present rates, the complete in-and-out round trip would take more than 30 hr. with the hole at its deepest, but it may be possible to achieve a factor of three with the new automatic hydraulic lifts that are now being developed. The life of a bit in very hard rock may be only equivalent to 50 ft. of drilling, so that there will possibly be a thousand trips in and out of the bore-hole. Even with the fast automatic pull-out, the time taken will be at least as long as that spent drilling.

It is possible, of course, that a modern version of the old cable-tool type of percussion drilling with its rapid method of pulling the tools out on a wire rope may be more suited to the hard basalt rock. Pneumatic chippers have been very effective in the Sahara recently in drilling tough siliceous limestone.

It is not unreasonable to consider even more fanciful means of cutting rock when standard methods are only capable of penetrating a few feet an hour. One such method is that of burning a hole by means of a flame torch. This is used for the 50-ft. type of hole that is drilled for shot-firing in mining. Suggestions have also been made that hard tough rock might be successfully broken up by letting off explosive charges at the bottom of the hole. The loosened debris could be removed afterwards by conventional drilling methods. A small charge of an ounce or so should shatter about 6 in. of rock, and if a technique could be devised to fire a shot every few minutes a drilling rate of 10 ft. or so an hour could be achieved.

It is quite clear that considerable research work is needed to produce a workable method of drilling the 50,000 ft. hole down to the Mohorovičić discontinuity, but the type of research work required is, in fact, already proceeding as part of the normal progress of oil-well drilling and rock mining. For example, the correct operation of the diamond cutter has been shown to depend on careful control of mud circulation and weight, and a factor of ten in distance of rock penetrated before the bit wears out may soon be possible. After all, diamond is tougher than the rock it cuts, and theoretically need not wear at all. In the opinion of most of those engaged in the industry, the problem of drilling to the Mohorovičić discontinuity could be successfully tackled now if the money for the project is forthcoming. The most promising method seems to be a combination of diamond bit with turbo-drill and fully automatic lifting mechanism, using sea-water circulation. The research effort to make the necessary improvements in the various components of this scheme would undoubtedly be repaid by speeding up normal oil-well drilling, just as motor racing has helped to produce better vehicles for everyday use.

The second deep drilling problem—that of drilling a few thousand feet through the sediments of the ocean bed—could be solved at once by using the techniques developed for underwater rock sampling off the California coast. Since 1953 a programme of geological investigation of this territory has called for many borings five or six thousand feet deep to determine the rock structures that exist beneath the sea-bed. In order to make these borings, a new technique was designed which permitted drilling from a floating vessel.

The motion of the vessel is taken up by gimbal systems and by sliding sections in the drill-pipe, so that drilling can proceed except in very rough weather. The barge is anchored to six mooring buoys and drilling is commenced through a string of casing suspended from the ship and clear of the ocean floor. After a few hundred feet have been drilled, the casing is cemented to the sea-bed, together with an assembly of valves and with guide-wires connecting to the ship, so that when the drill-pipe is pulled out it can be run back into the hole again.

In one trial a 300-ft. hole was drilled in a depth of 600 ft. of water without the complication of guide wires and well-head assembly at the sea-bed. The hole was drilled until the drilling bit wore out; then, since there were no guide wires it was impossible to find the hole again after pulling out to change the bit. However, it was possible to obtain samples of the rock that was drilled by cutting cores and pulling them to the surface in a cylinder lowered inside the drill-pipe on a wire line.

It is probable that this simple 'one-shot' technique would be adequate to drill through the soft sediments of the sea-bed to determine what is the underlying hard rock layer. There is very little wear on a coring bit when drilling clay, and the one bit should be able to penetrate all the soft material and at least a few feet of any hard layer. It may not be possible to anchor the drilling barge in 20,000 ft. of water, but it is

possible to anchor a marker buoy, and the barge could be kept continually in position relative to the buoy with the aid of tugs. It will be most important, of course, both to choose a place where good weather is probable, and to have good weather forecasts, because the main hazard of the operation will be the possibility of losing 20,000 ft. of drill-pipe.

There is no reason why this technique of drilling in deep water should not be extended by the use of the folding up, retractable drilling bit. If the bit can be changed without pulling the pipe from the hole, there is no limit to the depth. Experience may even show that the full Californian off-shore method is practicable, using casing cemented into the rock of the sea-bed and guide wires to the surface, in which case a very interesting possibility presents itself. The Earth's mantle is only about 10,000 ft. below the floor of the ocean, and if it is possible to drill continuously in deep water the task of reaching the Mohorovičić discontinuity would be much easier than even from a coral atoll. In the first place, it is only about 30,000 ft. below sea-level in the deep oceans, and in the second, the top 20,000 ft. is water which does not have to be drilled. The technical difficulties do not appear to be insurmountable, especially if the turbo-drill rather than rotary is used. It is probably largely a matter of gaining experience with the easier project of the two discussed, and selecting a site for the experiment where there is the essential good weather and negligible currents.

HIGH-SPEED FLIGHT

AT a time when the problems of the high-speed flight of aircraft, rockets and satellites are engaging the attention of scientists and engineers, it was very appropriate that Sections *A* and *G* of the British Association meeting in Glasgow on August 28 should devote one morning to lectures and discussion on this subject.

The subject was introduced by Prof. W. J. Duncan (Mechan professor of aeronautics and fluid mechanics, University of Glasgow), who underlined some of the vital principles behind these problems, showing that with the speeds that can now be attained the sharp distinction between the flight of aircraft and that of missiles has largely disappeared. The main engineering problems of high-speed flight can be grouped under four headings: (i) aerodynamic, (ii) kinetic heating, (iii) propulsion and (iv) guidance and control.

At supersonic speeds the shock-wave system around an aircraft causes an additional resistance (wave drag). One of the most important aims of the aircraft designer is to make this drag as small as possible. The problem of kinetic heating has given rise to the misnomer, 'the heat barrier'. The air in contact with the surface of the aircraft becomes heated, the effect being greatest at the most forward parts of the surface. Thus, when the speed of flight is 700 m.p.h., the temperature rise is 49° C., and this increases as the square of the speed.

The problem of propulsion depends upon the availability, or not, of atmospheric air. At extreme altitudes where the air density is very low, jet engines (for example, turbo-jets) can no longer be used and rocket propulsion has to be resorted to. Both jets and rockets have limited operational endurance (depending on the total fuel available); but whereas a turbo-jet gives a moderate thrust for a long time, a rocket gives a very large thrust for a comparatively short time.

Conventional controls are useless at altitudes where the air density is negligible. This can be overcome by using small jets to provide the moments required. The guidance of an unmanned missile may be provided by radar or by inertia navigation.

The subject was discussed in more detail in the two following lectures, the first of which, given by Mr. L. F. Nicholson (head of the Aerodynamics Department, Royal Aircraft Establishment, Farnborough), was on "Aerodynamic Aspects of High-speed Flight".

Mr. Nicholson defined the scope of the lecture as covering speeds "upwards from that of sound", the aim being to show the extent to which supersonic flight is becoming possible. For aircraft, one of the dominating factors in favour of high speed arises from the characteristics of jet engines, the thermodynamic efficiency of which increases considerably with speeds up to about five times that of sound. This increase in efficiency will be lost unless an efficient air inlet is used, which should also give low drag and avoid unstable pulsating flows; indeed, the air intake design can be one of the most exacting tasks of the design of a high-speed aircraft.

The aerodynamic efficiency was next considered. A measure of the efficiency of an aerodynamic shape for cruising flight is the ratio of the weight of the aircraft to the engine thrust required. Thus normal well-designed subsonic transport aircraft reach aerodynamic efficiencies of about 20. If such an aircraft were made to fly at supersonic speed by merely increasing the thrust, the efficiency would drop to about a tenth of its subsonic value (due to the unsuitable shape of the wings and fuselage for flight at supersonic speeds). This can be partially remedied by designing all parts of the aircraft to achieve full efficiency when operating in the flow field induced by the remaining parts of the aircraft; this has led to swept-back cambered wings and waisted fuselages. At speeds of the order of twice that of sound, a drop in aerodynamic efficiency is best avoided by using thin wing and tail surfaces and by drawing out the fuselage into a very slender shape. The wings themselves should be of large chord and of short span, with highly swept leading edges (having a plan form similar to that of a delta wing). The flow over such wings is highly complex, with separated flows springing from the leading edges at all incidences apart from that of cruising flight.

The kinetic heating problem mentioned above is mitigated to a certain extent by the effect of radiation, which increases rapidly with increase of temperature. By flying high, temperatures can be kept down to 600° C. at speeds five times that of sound.

Looking ahead to still higher speeds, a typical flight would consist of an accelerated climb (to a speed of the order of 10,000 ft. per sec.), followed by a long decelerating glide (for ranges of 2,000–3,000 miles), the aircraft being rocket powered. Sharp points and edges on the wings would have to be avoided as they would tend to become 'hot spots'. The energy of the fuel in such flights would be almost entirely used in building up the kinetic energy of the

aircraft ; thus a light weight would be more important than low drag. The weight of fuel used on a typical 3,000-mile flight need only be about twice that used in a cruising flight at supersonic speed ; thus hypersonic gliders are not beyond the realms of economic sense in the long-term future.

At these speeds longer ranges can be obtained in ballistic flight mainly outside the atmosphere. Here the problem of high temperatures and loads on re-entry to the atmosphere is indeed a frightening one. However, much of the kinetic energy of the motion could be diverted into heating the air in the wake rather than heating the aircraft. The best way to avoid excessive heating would be to allow the missile to follow a shallow trajectory on re-entry, using aerodynamic lift to increase the re-entry time from seconds to minutes. The increased time would allow radiation effects to dispel any excessive heating.

The final lecture, given by Mr. E. C. Cornford (head of the Guided Weapon Department, Royal Aircraft Establishment, Farnborough), was on "General Aspects of Travel at Very High Speeds with Special Reference to Rockets and Satellites". Mr. Cornford discussed the general propulsive and structural requirements of long-range ballistic vehicles and satellites, together with the problems of their guidance and control.

The flight pattern of a long-range rocket or satellite generally consists of a short period of powered flight during which the missile climbs out of the Earth's atmosphere. At the end of this stage the missile velocity ('all burnt' velocity) might be 22,000 ft. per sec. or more for a range of some 3,000 miles. The mass ratio (mass at launch/mass at 'all burnt') for such a single-stage rocket would be about 14. Thus only about 7 per cent of the initial weight of the missile is left for the payload, structure, motor, guidance and control systems, as well as any of the propellants which remain unused.

The position is much improved if a multi-stage rocket is used, that is, a missile in which two or more rockets are fired in succession, and as each burning stage is completed the empty propellant tank and propulsion unit are discarded. Thus a two-stage missile, each part having a mass ratio of 5, gives almost the same velocity as a single stage of mass ratio 25.

The main structural problem of a long-range rocket or of the launching vehicle for an Earth satellite is in the design of the integral propellant tanks, which are thin-walled cylindrical shells designed to withstand compressive end-loads (from the motor, missile inertia and drag), shear forces and bending moments (from the guidance system) and internal pressure. The thin walls of these tanks may be stabilized by internal pressurization or by mechanical means or by a combination of the two methods. The materials used need to have a high specific strength and stiffness and the ability to maintain these properties over a wide range of temperatures.

Much experience has been acquired relating to the use of liquid oxygen/kerosene systems. Possible improvements in rocket performance would be obtained by using various combinations of the oxidants (liquid oxygen, ozone and fluorene) and the fuels (liquid hydrogen, ammonia, hydrazine and the boron hydrides) to increase the specific impulse. It is questionable whether the gain in the specific impulse obtained in this way is worth the engineering effort required to make use of these liquids, almost all of which are either toxic or corrosive or liable to detonation. Recent developments with solid rocket propellants have led to specific impulses approaching those of current liquid propellants. Solid propellants greatly simplify the engineering problem, but the associated structure weights are appreciably higher. Nuclear rocket propulsion offers means of enabling space travel beyond the capabilities of conventional rockets.

The accuracy with which a long-range ballistic rocket will reach its target or with which an Earth satellite achieves a planned orbit will depend on its position and velocity at the end of its propulsion. For a ballistic rocket by far the most severe requirement is that for velocity accuracy along the trajectory at 'all burnt'. The guidance requirements for Earth satellites are likely to vary greatly with the purpose for which the satellite is intended ; at present any orbit within rather wide limits is a success.

Guidance of rockets or satellites consists of the determination of the position and velocity during its powered flight, and of computing the corrections to be made to the flight path to achieve the required terminal conditions. There are, in general, two types of guidance : (i) radar measurement of position and velocity, and (ii) measurement of acceleration by means of an inertia navigator in the missile. With radar guidance systems there is the problem of noise in the system (due to variations in motor thrust, in the measuring system itself and to ionization in the rocket flame). The centre of an inertia guidance system for a long-range rocket or a satellite is the space-stabilized inertia platform, carrying three orthogonally mounted accelerometers. The main problem with inertia guidance systems is in the design of gyroscopes and accelerometers of sufficient accuracy and stability of performance. The wander of the gyroscopes can be minimized by using a flotation gyroscope. The error characteristics of the radar and inertia guidance systems are to some extent complementary. The radar system has excellent long-term stability but suffers from noise at higher frequencies, whereas the inertia system has little high-frequency noise but suffers from long-term drift.

The main function of the control system of rockets or satellites is to apply the corrections given by the guidance system to the flight path. The couples necessary to change the attitude of the missile are usually produced by deflecting the efflux jets from the main motors or by movable vanes in the jet. The control problem is complicated by any flexibility of the structure and by fuel sloshing.

In the subsequent discussion many points were raised in connexion with re-entry into the atmosphere. One speaker thought that re-entry to the denser air should be achieved as quickly as possible, the resulting heated external surface being effectively insulated from the interior of the missile. As pointed out by Mr. Nicholson, this would be doubtful from a practical point of view. Heat transfer is more severe under normal flow conditions. Powered deceleration is ruled out because of the additional fuel required. Re-entry by 'skip' would be of advantage in giving more time for the cooling of the missile but would create many problems in guidance and control.

The audience were left with the impression that there are no insurmountable technical barriers to be encountered in this field, although the practical difficulties of attaining the objectives seem formidable.

A. W. Babister

OBITUARY

Dr. Malcolm A. Smith

Dr. Malcolm Arthur Smith, a well-known authority on amphibians and reptiles, died at his home at Ascot on July 22 at the age of eighty-two.

He qualified M.R.C.S., L.R.C.P. in 1898 at Charing Cross Hospital and remained there for four years as house physician and surgeon before going to Siam, where he took up an appointment as medical officer to the British Legation in Bangkok. For almost a quarter of a century he practised medicine in Bangkok, during which period he acted as physician to the Court of Siam. He was one of the very few Europeans to come in contact with life at the Court and to have first-hand knowledge of its organization. On his return to Britain he wrote an interesting account of the remarkable happenings in the lives of the royal family which was entitled "A Physician at the Court of Siam", published in 1947.

His interest in herpetology gathered momentum as his zoological expeditions to the interior of Siam, Indo-China and Malay produced more rich and valuable collections, and he devoted more and more of his leisure time to the study of the Oriental species. He was an acute observer and a prolific writer, having more than a hundred publications to his credit. Most of these were based on classification and structure, although, more recently, he turned to considerations of evolutionary changes in the ear and the eye-covering of lizards, in snake dentition and in the head glands of snakes. His taxonomic studies reached a climax in an outstanding monograph on sea snakes and in the three volumes on the crocodiles and tortoises, the lizards, and the snakes, published in the "Fauna of British India" between 1931 and 1943 and after he had retired from Siam and had become an honorary associate of the British Museum. During the last decade or so of his life he committed himself to a study of the reptiles and amphibians of Great Britain, acknowledging with a wide comprehension the problems that were still unsolved or unpublished in that field. He became the author of yet another outstandingly successful book, "The British Amphibians and Reptiles", which was immediately recognized as a comprehensive and thoroughly reliable account of the structure, habits and behaviour of our British species. This is a book that is an invaluable guide to the specialist besides providing enjoyment to the layman, and it has become the standard work on the subject.

Not only an account of his scientific works shows him to have been an experienced and energetic personality, but the posts he held also indicate the zeal and devotion he gave to the advancement of herpetology. He was the first president of the British Herpetological Society from its inauguration in 1947 until 1954, recorder for the Amphibia and Reptilia section of the "Zoological Record" from 1929 until 1945 and editor from 1938 until 1949, and zoological secretary to the Linnean Society from 1939 until 1948.

A. G. C. Grandison

NEWS and VIEWS

Commonwealth Advisory Committee on Defence Science

A meeting of the Commonwealth Advisory Committee on Defence Science is to be held in Australia during October 6–28. Sir Leslie Martin, defence scientific adviser to the Australian Department of Defence, will be the general chairman of the meeting. The Australian delegation will be led by Mr. W. A. S. Butement, chief scientist of the Department of Supply, and other delegations include Dr. J. E. Keyston, vice-chairman, Canadian Defence Research Board; Dr. D. S. Kothari, scientific adviser to the Indian Minister of Defence; Dr. Mohammed Afaf, Pakistan; Dr. S. M. Naude, scientific adviser, Department of Defence, South Africa; Sir Frederick Brundrett, scientific adviser to the United Kingdom Minister of Defence, Air Marshal Sir Geoffrey Tuttle, Deputy Chief of the Air Staff, and Dr. D. H. Black, director, Armament Research and Development Establishment. New Zealand will also be represented. These meetings are held at approximately three-yearly intervals and the last was in Canada in February 1956. The object of the Committee is to promote collaboration throughout the Commonwealth in the application of science to military affairs, and the meeting will be attended by senior defence scientists and senior officers of the armed forces of the Commonwealth.

Imperial Cancer Research Fund

Dr. J. Craigie has resigned his post as director of the Mill Hill laboratories of the Imperial Cancer Research Fund so that, free from administrative duties, he can devote himself entirely to research in the laboratories. His invaluable advice on problems of research will thus continue to be available to the Fund. Dr. R. J. C. Harris is joining the Fund's scientific staff on October 1 to take up a new appointment as head of the Division of Experimental Biology and Virology. He will also be responsible (under the director, Dr. G. F. Marrian) for the general administration of the Mill Hill laboratories. Mr. A. R. Currie, at present assistant pathologist at the Royal Infirmary, Glasgow, has been appointed head of the Division of Pathology, and will take up his duties on October 1, 1959. The Fund is meanwhile giving financial support to the cancer research he is undertaking in the Pathology Department of the University of Glasgow.

Transfer of Controlled Thermonuclear Project from Harwell

It is proposed to remove the whole of the controlled thermonuclear project from the Atomic Energy Research Establishment, Harwell, to Winfrith Heath, Dorset. This change will permit the development of both fission and fusion work at Winfrith, and allow room for whatever expansion of the latter is necessary in the future. It will also provide a margin for new work as yet unseen which will undoubtedly be developed at Harwell. Winfrith Heath, which is near Dorchester, is a new site taken over by the United Kingdom Atomic Energy Author-

ity. When first announced it was said that it would be a "second Harwell" and that it would provide scope for work on reactor development. Development of the site started in September 1957, and one of the first projects is the construction of a high-temperature gas-cooled reactor.

British Ballistic Missile fired from Woomera

A British 'Black Knight' ballistic missile was successfully launched to a height of 300 miles from Woomera rocket range on September 7. The Australian Minister of Supply, Mr. Atholl Townley, who was at Woomera to see the launching, is reported to have said that the rocket performed according to expectations. The 'Black Knight' is a purely research vehicle, and observations of the behaviour of the rocket engine, control and guidance mechanisms, together with measurements of its environment and other phenomena, were made continuously while it was in flight and relayed to ground receivers from several transmitters installed in the missile. Its behaviour during re-entry into the Earth's atmosphere was also recorded; the nose-cone has been recovered. The 'Black Knight', which is about 35 ft. long and 3 ft. in diameter, was largely developed and built by Saunders-Roe, Ltd., in co-operation with the Royal Aircraft Establishment. Static firings were made at Highdown, on the western tip of the Isle of Wight. The rocket engine was built by Armstrong-Siddeley Motors, of Coventry, to an original design of the Rocket Propulsion Department of the Royal Aircraft Establishment at Westcott.

Swedish National Committee for Physics

The ninth general physics conference of the Swedish National Committee for Physics was held at the University of Uppsala during June 3–5, 1957, and a brief report of the meeting together with abstracts of the large number of contributed papers is printed in the March issue of *Arkiv für Fysik*. (13, 251; 1958). Guest lectures were given by M. A. Melvin (Florida State University), who spoke on conservation-invariance principles and the behaviour of elementary particles, and by H. Palevsky (Brookhaven National Laboratory), who discussed the use of cold neutrons to investigate the solid state.

The majority of the contributed papers deal with aspects of nuclear physics, but there were several survey articles or lectures of general interest. H. Conde discussed the generation and measurement of high thermal radiation intensities, and E. Djurle and W. S. Rodney instrumentation for phase contrast measurements in the near infra-red. A brief survey of present-day knowledge of the elementary particles and of their transformations was given by S. von Friesen, and an account of the proposed new physics building for the University of Stockholm, which is expected to be ready for use in 1959/60, by E. Hulthen. A description of the Uppsala Ionosphere Observatory was given by W. Stoffregen. Its geographical situation is near the auroral zone and instruments for ionospheric research and an all-sky aurora camera are in operation at the observatory. The ionospheric recorders, or 'ionosondes', designed and constructed at the observatory, consist of a radio pulse transmitter and receiver, together with oscillograph units for photographic recording of the radio echoes, and they make vertical soundings of ionized layers of the atmosphere. For study at oblique incidence by means of backscatter, a rotating beam antenna is used and reflexions up to 2,000 mk. distance can be received. Since January 1957, a secondary station has been in action at Lycksele, about 500 km. north of Uppsala, and six aurora cameras are also operating at places between Uppsala and Tromsö.

Institution of Metallurgists Handbook

During the first quarter of the present century, more metal was produced and used throughout the world than during the whole of previous historic time. This growth in the practice of metallurgy demanded a body of trained and qualified metallurgists. It is, however, barely a dozen years ago since the Institution of Metallurgists was founded to meet this need. Its first object is "to advance and co-ordinate the study and science of metallurgy in all its aspects". In so doing, the Institution clearly serves the public interest, not only by certifying the scientific or industrial qualifications of its members, but also by its work, for example, in connexion with National Certificates in its subject. That a need for such an Institution existed and that it is serving the purpose for which it was founded is abundantly demonstrated by the growth of its membership, a growth which surprises even the most sanguine of its founders.

In its new form, the Year Book and List of Members for 1957–58, published for the Institution by Batiste Publications, Ltd., gives expression to the increasing prestige which the Institution enjoys and deserves. In addition to the list of officers and members and the conditions under which admission to the various grades of membership may be obtained, 75 pages are devoted to short technical articles dealing with the more important of the individual metals. In the space available these articles cannot, of course, be exhaustive, but they are very far from being superficial and the data contained are authoritative. In a foreword, Mr. James Mitchell, the president at the time this handbook was prepared, summarizes the aims of the Institution, aims which in a large measure, at any rate, have been attained, in the following words: "The Council of the Institution are most anxious that its membership should include all practising metallurgists who have the necessary qualifications, but they are insistent that a high professional standard must be maintained and they take this to include not only technical qualifications and experience but the observation of high ethical standards".

Careers in the Medical Research Council

Under the title "Technical Careers in the Medical Research Council" (pp. 31. London: Medical Research Council, 1958), the Medical Research Council has issued a brochure giving a concise but clear picture of the opportunities which are open as technical officers or technicians in the service of the Council. The Council's staff of 2,152 (only 121 of whom are part-time) comprises 224 medically qualified, 424 scientifically qualified and 91 technical officers, with 690 other technical staff, 332 maintenance and 391 administrative and clerical staff. Besides information about the general conditions of service, a brief survey of the types of work undertaken in the Council's establishments and a note on the qualifications required for entry to the various grades, the booklet includes a survey of the research programme under the main scientific headings and an alphabetical list of the Council's establishments and their addresses, which make it useful to a wide range of scientific workers.

Technical Authorship

A SCHEME of instruction and examination in technical authorship, intended for those with engineering, technical or scientific training and experience, has been drawn up by the City and Guilds of London Institute Advisory Committee with the needs of the younger entrants in mind. The examinations will also be open to practising technical authors. The regulations and syllabuses are detailed in a pamphlet, "Technical Authorship", issued by the Institute (pp. 9. London: City and Guilds of London Institute, Gresham College, 1958. 9*d*.). A four-year part-time course of instruction is envisaged and the Intermediate examination of the Institute will be taken at the end of the second year and the Final examination at the end of the fourth year of the course.

Queen Victoria Museum and Art Gallery, Launceston

THE report for 1956–57 of this progressive Museum and Art Gallery records that the renovation programme has been continued. The main projects have been the complete reconstruction of the china room, the construction of new cases in the vertebrate gallery, improvements in the lecture room and additional storage accommodation. In association with the University of Tasmania, an expedition to examine the rock markings in the Blue Tier district was undertaken. It is suggested that these are aboriginal in origin. Also in association with the local field club, an exhibition of native plants was arranged and maintained for about three months. The engagement of a temporary assistant in the geological department produced considerable development in this sphere, and many improvements were effected.

The *Records of the Queen Victoria Museum*, New Series No. 9, includes an important paper on the Van Diemen's Land Company 1825–1842 by Mr. A. L. Meston (pp. 62. 1958. Launceston, Tasmania: Queen Victoria Museum). From a study of the wool trade in Britain and the increasing inadequacy of the supply of fine wool the work proceeds to the chartering of the Van Diemen's Land Company in 1825. Its grant was for districts outside the settled areas and its power confined to producing fine wool. The colonists, however, were suspicious of absentee monopolists, and the regions of land granted were quite unsuited to raising fine-woolled sheep. Stock losses were heavy from weather, wolves and aborigines. Finally, the management were unable to overcome the natural and official obstacles, and the Company closed in 1842. The paper forms an important side-issue of the early history of the wool trade in England.

Stable Graticules

MESSRS. GRATICULES, LTD., have recently introduced a new graticule process which they term 'maXta' (for maximum stability). It has the advantage that the graticule is composed solely of a noble metal on glass; there is no gelatine or other support material. The image is firmly bonded to the glass and is extremely resistant to abrasion, obviating the necessity for a cover glass. This is an important consideration, since breakdown in graticules is often associated with the cover-glass cement. Further, the absence of gelatine means that there is no suitable substrate for fungal attack—an advantage in tropical conditions. The method is based upon work commenced by the late Julius Rheinberg. It is essentially a photographic process and is most suitable for lines on a transparent ground rather than the reverse. The technique can be applied to most of the available glasses. Three types are made having varying optical qualities. These variations are associated with the adhesion rather than with the image quality, which in each case is very good. These graticules were developed in the course of work carried out at the British Scientific Instrument Research Association, Chislehurst, Kent.

Germination of Oil-Palm Seed

THE results of an interesting investigation of the factors controlling seed germination in the West African oil palm, *Elaeis guineensis* (Jacq.) have been given by G. Hussey (*Annals of Botany*, N.S., 22, 259; 1958). It is recorded that under natural conditions in West Africa, at the average ground temperature of 25–30° C., oil-palm seed germinates sporadically over a period of years, but with continuous heat treatment (38–40° C.) 50 per cent germination of either nuts or extracted kernels occurs within a few months. The kernel comprises a small cylindrical embryo embedded in a mass of oily endosperm which in turn is surrounded by a thick testa. At germination the testa and layer of endosperm covering the distal part of the embryo are forced out as a disk-like operculum which is already demarcated in the ungerminated kernel by a circular abscission zone in the endosperm cells. The excised embryo is non-dormant and at 30° C. starts to elongate within 24 hr., either on moist filter paper or while still in contact with the endosperm. At 30° C. removal of the operculum is followed by a sluggish emergence of the embryo in air; emergence at the normal rate occurs in pure oxygen, or in air if a long period at high temperature precedes deoperculation. Fresh intact kernels remain ungerminated for at least six months at 30° C., either in air or in pure oxygen. At high temperature (40° C.) intact kernels germinate faster in pure oxygen than in air; at 30° C. the same applies only after previous treatment at high temperature. This accelerating effect of oxygen is shown to be dependent upon the progress of slow processes stimulated by high temperature. It is postulated that germination of oil-palm seed is dependent upon a minimal threshold concentration of oxygen in the embryo and that during dormancy this minimal level progressively decreases at a rate depending on the temperature.

Harkness Fellowships of the Commonwealth Fund

THE awards which have borne the title of Commonwealth Fund Fellowships since 1925 are being offered for 1959 under the title of Harkness Fellowships of the Commonwealth Fund. The Fund is an American philanthropic foundation endowed by the late Mrs. S. V. Harkness and the late Mr. and Mrs. E. S. Harkness, for promoting international understanding by providing opportunities for education and travel in the United States. Fellowships tenable in the United States are offered in separate series in the United Kingdom, in Australia and New Zealand and in Western Europe. Thirty Fellowships are offered in the United Kingdom for 1959 to men or women who have not previously worked for more than a few months in the United States, and who are graduates or have experience in government service, the professions, the creative arts, journalism, branches of business or industry. Forms of applica-

tion, which must be returned before December 2, can be obtained from the Warden, Harkness House, 38, Upper Brook Street, London, W.1, from whom further details can also be obtained.

Atom School for Business Men

Nearly three hundred company directors and similar executives have been to four-day courses organized by the Isotope School, Atomic Energy Research Establishment, Harwell, during the past year. These courses are designed to give delegates information about current and possible future uses of radioisotopes in industry. The Isotope School at Harwell is running a further course during September 23–26 which is open to any company director or similar executive. There are still a number of places available on this course. Delegates will be housed at Worcester College, Oxford, for four nights and taken daily to Harwell. There will be lectures and demonstrations and visits arranged to atomic reactors, the Isotope Division Handling Hall and the Wantage Radiation Laboratories, where research is directed towards initiating the commercial use of large-scale irradiation sources. Further information can be obtained from the Registrar, Isotope School, Atomic Energy Research Establishment, Harwell, Didcot, Berks.

International Conference on Information Processing

An international conference on Information Processing will be held in Paris during June 15–20, 1959. The conference is being organized by Unesco with the help of a group of consultants from various countries. Persons who wish to offer a paper should send the title, with three copies of an abstract of about 250 words, to their national group before October 1. Abstracts must be submitted in English or in French (or be accompanied by a translation in one of these languages). Further information can be obtained from the Honorary Secretary, Group *B*—Computation and Automatic Control, British Conference on Automation and Computation, c/o The Institution of Electrical Engineers, Savoy Place, London, W.C.2.

University News : London

Dr. R. C. Curran, senior lecturer in the Department of Pathology of the University of Sheffield, has been appointed to the University chair of pathology tenable at St. Thomas's Hospital Medical School. Mr. M. de Burgh Daly, Locke Research Fellow of the Royal Society in the Department of Physiology at University College, has been appointed to the University chair of physiology tenable at St. Bartholomew's Hospital Medical College. Dr. P. L. Pratt, lecturer in physical metallurgy in the University of Birmingham, has been appointed to the University readership in physical metallurgy tenable at the Imperial College of Science and Technology. Dr. A. M. Cook, senior lecturer in pharmaceutics at the School of Pharmacy, has been appointed to the University readership in pharmaceutics tenable at that School.

Scientific Film Association

Owing to the continued growth in membership and the consequent increased demands on its services, the Scientific Film Association has moved to new offices in 3, Belgrave Square, S.W.1. These premises have been made available by Aslib, and it is anticipated that this closer relationship will introduce the work of the Scientific Film Association to many who are concerned with documented information of all kinds.

Announcements

Dr. Joseph Auer, head of the Department of Anatomy, Faculty of Medicine, University of Ottawa, has also been appointed assistant director of the Division of Medical Research, National Research Council of Canada, from August 1.

Prof. H. C. Darby has been appointed to fill the vacancy in the membership of the National Parks Commission created by the retirement of the Rev. H. H. Symonds.

Mr. N. S. Billington, who has been head of the National College for Heating, Ventilating, Refrigeration and Fan Engineering since February 1950, has resigned as from August 31, 1958, to take up the appointment of director of the Heating and Ventilating Research Council. Dr. David R. Scott, at present a senior lecturer on the staff of the Royal College of Science and Technology, Glasgow, has been appointed to succeed Mr. Billington.

The Corday-Morgan Commonwealth Fellowship for the year 1958/59 has been awarded to Miss Rama Bhattacharya, who has, since January 1957, been junior research fellow of the Council of Scientific and Industrial Research, India, at the University College of Science, Calcutta. It will be tenable in the Department of Chemistry, University College, London, under Prof. Sir Christopher Ingold. The Fellowship is not offered for the academic year 1959/60.

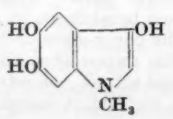
A conference on "Food and Farming : a Survey of Problems and Remedies" will be held, under the auspices of the Association of Agriculture, at the London School of Economics, on September 27. Further information can be obtained from the general secretary, The Association of Agriculture, 53 Victoria Street, London, S.W.1.

The Scottish Group of the Nutrition Society is holding a symposium on "Nutritional Hazards caused by Radioactive Contamination of Foods" in Edinburgh on September 27. Those interested, who are not already members of the Society, are invited to write for further particulars to Dr. J. Davidson, the Rowett Research Institute, Bucksburn, Aberdeenshire.

The Institute of Personnel Management is to hold a conference on "The Individual and the Organization in Modern Society" at Harrogate during October 10–12. Further details can be obtained from the Institute at Management House, 80 Fetter Lane, London, E.C.4.

A meeting of the Photoelectric Spectrometry Group will be held in Courtauld Hall, Queen Elizabeth College, Campden Hill Road, London, W.8, on October 17, at 2.30 p.m., when three papers dealing with spectropolarimetry will be read. Further information can be obtained from Mr. K. A. Macdonald, Arbury Works, 56, Arbury Road, Cambridge.

Erratum. In the communication entitled "Reduction of Adrenochrome with Ascorbic Acid", by Dr. R. A. Heacock and Miss B. D. Laidlaw, in *Nature* of August 23, p. 526, there is a misprint in formula V (adrenolutin) ; it should be



THE COUNCIL FOR NATURE

THE inaugural meeting of the Council for Nature took place in the rooms of the Zoological Society of London on July 12, when Lord Hurcombe delivered his presidential address. He emphasized that the Council has no intention of taking up an impracticable or hostile attitude towards developments necessary for the economic interests of Britain, but that nevertheless the preservation of natural life and resources is entitled to a high place in a civilized and highly populated country. Secondly, he felt that schemes involving biological devastation should be more seriously investigated than they sometimes were, and that necessity rather than convenience should be borne in mind. Further, while local and specialized societies are often engrossed with their restricted interests, they have a duty towards securing a state of public opinion that considers the interests of naturalists realistically. Indiscriminate chemical warfare on pests and river pollution by effluents show that a full realization of the problems has certainly not yet been achieved.

Lord Hurcombe appealed to all interested societies and authorities to join in making a concerted attempt to preserve the remaining resources of natural life in Britain, and gave assurances that the Council would encourage the establishment of Nature reserves and naturalists' trusts, and would co-operate so far as possible with the Society for the Promotion of Nature Reserves and with the Council for Field Studies. Finally, he announced that the Council for Nature is to have the honour and stimulus of the patronage of H.R.H. the Duke of Edinburgh. The meeting then proceeded to consider items of essential business. The draft constitution was adopted and the members of the executive committee elected.

The chairman, Sir Landsborough Thomson, described the functions of the three sub-committees that the Council proposes to set up. One will deal with conservation problems; another will concern itself with education; and the third will be for publicity and public relations.

Mr. Aubrey Buxton, honorary treasurer, then dealt with the proposal that a magazine should be published for the Council. This would certainly not be a house journal, but a popular journal aimed at a wide public with an interest in many aspects of natural history and the countryside.

One of the most important aims of the Council is to create a new interest in the countryside among school children and organized youth groups. Some of this interest could be guided into practical work in Nature reserves and other conservation projects.

After the business meeting, Mr. Tony Soper spoke of the work of the Natural History Unit of the B.B.C., explaining, with illustrations, many of the problems involved in filming and making sound recordings for natural history programmes. The secretary of the Council, which is now supported by more than a hundred societies, is Mr. D. J. B. Copp, 41 Queen's Gate, London, S.W.7.

THE ROYAL SOCIETY OF CANADA

THE seventy-sixth annual meeting of the Royal Society of Canada was held at Edmonton, Alberta, during June 2–4, under the presidency of Prof. Thomas W. M. Cameron, of McGill University. In celebration of the jubilee of the foundation of the University of Alberta under the presidency of the late Dr. H. M. Tory in 1908 and because Edmonton is the gateway to the north-west of Canada, a symposium was held on the "Potentialities of the North-West". The subject was introduced by Dr. C. H. Mackenzie, president of the Atomic Energy Control Board and past-president of the National Research Council of Canada. He was followed by Dr. R. F. Legget, director of building research, National Research Council, who discussed transportation and housing possibilities; by l'Abbé Antoine d'Eschambault, who dealt with the historic background; by Prof. W. C. Wonders, of the University of Alberta, who considered geographical problems; and Prof. D. Rawson, of the University of Saskatchewan, who spoke on biological problems of the region. The symposium was closed by Dr. D. B. Turner, Deputy Minister of Recreation and Conservation and Commissioner of Fisheries, British Columbia, who summed up the possibilities of northern British Columbia, the Yukon and the Mackenzie Valley.

Sectional symposia were also presented on "Oceanography" and on "Physical Methods in Organic Chemistry". In addition, papers were read in the various Sections of the Royal Society of Canada: mathematical, chemical, physical (under the presidency of Dr. Leo Marion); geological (under the presidency of Mr. H. C. Rickaby); and biological (under the presidency of Dr. W. R. Campbell).

Prof. Cameron's presidential address was on "The Evolution of Evolution". He noted that in the year of the founding of the University of Alberta, the Linnean Society had awarded Alfred Wallace a medal for the paper read fifty years previously, simultaneously with Charles Darwin, when the two naturalists introduced the subject of evolution by natural selection. Wallace had named the subject "Darwinism" and had left the field free for Darwin to develop further. As a result, Wallace's part was largely minimized or overlooked. Another man whose work had been misinterpreted, mistranslated and, to a great extent, overlooked, was Lamarck—discredited by Cuvier in France and both misrepresented and ignored elsewhere. It seemed obvious that few workers had read his original "Zoological Philosophy"; if they had, his part in the evolution of the theory would be much better understood. Other workers whose views had played a part in preparing the way for Darwinism were Buffon and Erasmus Darwin—the remarkable grandfather of Charles—as well as the geologists Hutton and Lyell. Prof. Cameron quoted Darwin's own views on this: "Species have been modified during a long course of descent chiefly through the natural selection of numerous successive slight, favourable variations: aided in an important manner by the inherited effects

of the use and disuse of parts, and in an unimportant manner (that is, in relation to adaptive structures whether past or present) by the direct action of external conditions and by variations which seem to us in our ignorance to arise spontaneously. . . . I am convinced that Natural Selection has been the most important but not exclusive means of modification".

Prof. Cameron said that while Wallace gave a stimulus to Darwin to publish "The Origin of Species" in 1859, much of its immediate popularity was due to Herbert Spencer and Thomas Huxley. The theory of natural selection stated a fact and did not attempt to explain how the variation arose—merely how it was used. The kernel of the theory lay in heredity, and Mendel, almost simultaneously with the Wallace–Darwin papers a century ago, was laying the foundations of genetics to explain how interspecific variation was inherited. However, much still remained to be discovered, particularly the part played by the cytoplasm of the germ cells in producing major fundamental changes. The year 1958 marks not only the centenary of the Wallace–Darwin publication and the bicentenary of the tenth edition of the "Systema Naturæ" of Linneus, but also the century of the publication of the first description of Neanderthal man which directed attention to the fact that the evolution of man himself is the most important aspect of the theory.

Civilization represents the modern evolution of man and civilization has to grow and change, or decay and die. Growth and change do not always mean progress; in fact, the history of life on Earth shows that more often they lead to extinction. Man is the first and only animal, however, that has some control over his environment. Used wisely, that control can lead to progress.

"The constitution of the heavenly bodies and the structures of the atom," said Sir Oliver Lodge, "are in a sense elementary compared with the problems which confront those who would ascertain and disentangle even the material aspect presented by living things."

The following were elected to office in the Royal Society of Canada for the year 1958–59: *President*, M. Pierre Daviault; *Vice-President*, Dr. H. G. Thode; *Honorary Secretary*, Col. C. P. Stacey; *Associate Honorary Secretary*, Dr. Guy Sylvestre; *Honorary Treasurer*, Dr. N. E. Gibbons; *Honorary Editor*, Dr. G. W. Brown; *Honorary Librarian*, Dr. W. Kaye Lamb; *Editor of 'Studia Varia' for* 1959, Dr. T. W. M. Cameron; *President of Section* 3 (*Mathematical, Chemical and Physical Sciences*), Prof. G. M. Shrum; *President of Section* 4 (*Geological and Allied Sciences*), Dr. L. S. Russell; *President of Section* 5 (*Biological Sciences*), Dr. N. H. Grace.

FODDER PLANTS

A SYMPOSIUM on fodder plants, organized by the Institute of Plant Breeding of the Polish Academy of Sciences, was held at Poznań during June 25–27. It was attended by delegates from the U.S.S.R., the United States, Sweden, Germany, Hungary, Czechoslovakia and the British Commonwealth, who later had the opportunity of inspecting the experimental fields at the research stations of Baborówko and Przebedowo, near Poznań, and a number of other experimental stations farther afield. One of the most striking features of the symposium was that, although several of the contributions dealt with clovers, lucerne and other fodder plants, the great majority were devoted to lupins. Many areas of Poland have light sandy soils where lupins are among the few leguminous species that can be grown successfully. Mineral fertilizers seem to be in rather short supply, so that lupins are encouraged for the sake of their beneficial effects on soil fertility. Some are used as green manures. The greatest attention is, however, focused on the so-called sweet lupins which, owing to their low content of bitter alkaloids, can be used as fodder for livestock. The sweet lupins were first produced in Germany, and during the war years breeding work was carried out with them at Puławy in Poland. From this material hundreds of promising selections have now been produced and were demonstrated in the experimental fields. The most successful are selections of *Lupinus luteus*, which are more reliable in yield, but promising sweet selections of *L. albus* and *L. angustifolius* were also shown. The selections were distinguished by greater vigour and yield of green matter, earlier maturity, freedom from pod shedding, greater palatability, smaller seeds and other features.

By a group of investigators working at various research stations in Poland an intensive study has been made of the chemical structure of the lupin alkaloids, and many of the Polish contributions to the symposium dealt with these studies, largely based on analysis by paper chromatography. Several alkaloids not hitherto identified have been found, one of which has been named angustifolin, having been detected first in *Lupinus angustifolius*. On the basis of the structure of their alkaloids E. Nowacki (Poznań) has classified the genera of the family Genisteae, and the conclusions correspond with many arrived at on purely morphological features. Thus, *Laburnum* never contains spartein, its alkaloids, consisting of cytisin, methylcytisin and laburnin, whereas many *Cytisus* species contain spartein but no laburnin. The separation of the two genera is thus justified. *Sarothamnus* contains no cytisin and should be excluded from the genus *Cytisus*. In the same way the relationships between the species within the genus *Lupinus* have been assessed, and the grouping arrived at is borne out by considerations of the composition of the anthocyanins, flavones, proteins and fats and corresponds to the differences in chromosome number where these exist. A triple hybrid of (*L. ornatus* × *L. mutabilis*) × *L. douglasi* was described, some of the segregates being distinguished by high yield and earliness.

Papers presented by delegates from abroad included a review of the use of polyploidy in plant breeding in Sweden by A. Hagberg (Svalöf), who referred to three tetraploid clovers, one of rye and one of swede turnip produced at Svalöf; Ulva red clover exceeds the diploid by 11 per cent in yield, and Sirius swede exceeds the diploid by 16 per cent while having a dry-matter content of 12 per cent.

N. A. Majsurjan (Timirjazev Academy of Agriculture, Moscow) spoke of experience with sweet lupins in the Soviet Union. *Lupinus angustifolius* and the sweet forms of *L. albus* and *L. luteus* have large seeds, and efforts are being made to produce forms

with small seeds. Sweet forms of *L. polyphyllus* are of interest, particularly on account of their earliness. From a cross of *L. angustifolius* × *L. linifolius* a promising form has been selected which combines the small seeds of the latter species with a plant type similar to *L. angustifolius*, together with earliness and low alkaloid content. Evidence was presented in support of the view that the species *L. mutabilis*, which crosses easily with the small-seeded American species such as *L. ornatus*, is itself a hybrid.

H.-J. Troll (Müncheberg) spoke of experiments on producing sweet forms of *L. luteus* and *L. angustifolius* which ripen uniformly and do not shed their seeds. Some useful mutants have been obtained by irradiation with X-rays, and latterly also with cobalt-60. J. Hackbarth (Max Planck Institut, Scharnhorst) spoke of mutants characterized by vigorous growth and others with a thermoneutral developmental reaction, suitable for growing in northern latitudes or for late sowing. W. Plarre (Hadmersleben) referred to experiments in which such thermoneutral types could be detected by vernalization and described a promising new hybrid produced by this means which is being released for cultivation in Eastern Germany. Several speakers referred to impressions that the sweet strains, especially those free from shedding, are inferior in yield, but evidence was given by Prof. Hackbarth and others to show that this is not necessarily so.

Contributions dealing with topics other than lupins included an account by N. Clark (Agricultural Experiment Station, Madison, Wisconsin) of the co-operative research on forage production leading, after twenty-five years of work, to the production of the first synthetic lucerne variety, Vernal. A detailed description was given by E. Åkerberg (Svalöf) of the development of the seed-production industry in Sweden, as a result of which Sweden now produces most of her own seed of the main species of forage plants. Among the achievements of plant breeding, special mention was made of the red clovers resistant to *Sclerotinia* and eelworm, the winter-hardy lucernes, the persistent and high-yielding white clovers, the polyploid clovers and strains with greater leafiness, better powers of a series of improved grass recovery and superior quality.

R. Focke (University of Rostock) mentioned some tetraploid red clovers that are 36 per cent better than diploid strains in fodder yield. They are, however, 20–40 per cent lower in fertility. Certain other speakers mentioned the same difficulty, though in certain cases a fairly satisfactory seed set had been obtained; thus a figure of 20 per cent was mentioned by H. Kress (Germany) for certain tetraploid red clovers, and up to 89 per cent in tetraploid white clover after selection for short corolla tube.

P. S. Hudson

SCIENTIFIC RESEARCH AND DEVELOPMENT IN EAST AFRICA

THE annual report of the East Africa High Commission for 1957*, in which a foreword by the chairman, Sir Evelyn Baring, records the tenth anniversary of the establishment of the High Commission, notes also the concentration of its eight departments in new headquarters in Upper Hill Road, overlooking the city of Nairobi. Almost half the report deals with the research and scientific services. The East African Agriculture and Forestry Research Organization records steady progress, and stress is laid on the value of the work on the full utilization of the inadequate or unreliable rainfall and on the chemistry of soil fertility. A statistical study of published data on the digestibility of the protein fraction of fodders indicated that all ruminants have the same ability to digest the protein in fodders, and that its digestibility in normal fodder depends solely on the protein level in the ration and not on its sources. The East African Veterinary Research Organization records further significant advances in the growth in cell culture of various types of rinderpest, Rift Valley fever and sheep pox, and the agar double diffusion precipitation test has been applied in the diagnosis of pleuropneumonia, while a co-operative project with visiting scientists from the United States is concentrating on African swine fever in the first instance.

The East African Fishery Research Organization has made extensive studies on the food of commercially important species, but its hydrological observations, which require extension, have been handicapped for lack of a vessel which can be used for prolonged journeys across Lake Victoria. The East African Marine Fisheries Research Organization has been provided with a new research ship, and the Organization has been able, in consequence, to extend its programme to include tuna long-line fishing on a greater scale. Under the East African Trypanosomiasis Research Organization different methods of collecting *G. pallipedes* have been studied in south-east Uganda as part of a fundamental study of the density of flies and their infection-rates. Work on trypanosome immobilization and complement-fixation tests continued, and methods have been developed for deep-freezing *T. brucei*, *T. rhodesiense* and *T. congolense* for up to seven months with solid carbon dioxide. Work on the trypanosomes in the fly has shown that the infection does not reduce the life of the fly. Physiological studies on tsetse flies are also reported.

Much of the work of the East African Institute of Malaria and Vector-borne Diseases is advisory; but its research activities have included studies of the life-cycle of anopheles, an experiment on the control of malaria in the Parc–Tavetz area, and bilharzia studies. The East African Virus Research Institute was concerned mainly with the natural history of the insect-borne virus diseases in East and Central Africa, but clinical work has now been started. The East African Institute for Medical Research continued its work on the absorption of foodstuffs, and the pilot scheme for eradicating filariasis on Ukara Island. The main work of the East African Leprosy Research Centre during the year was the therapeutic trial of diphenylthiourea started in 1956. The Lake Victoria Fisheries Board reports evidence that the species *Tilapia zillii* may help to offset the annual dearth of fish in the off-season of July. The East African Industrial Research Organization continued to investigate the artificial drying of Arabic coffee and is now providing an advisory service in fuel technology, while its work on the development of a sound technique for producing vitreous white ware pottery is

* Annual Report of the East Africa High Commission for 1957. Pp. x+85. (London: H.M. Stationery Office, 1958.) 7*s.* 6*d.* net.

emphasized. Expansion of the East African Meteorological Department is planned and promising results were obtained in work on the reduction of evaporation of water from reservoirs with a film of cetyl alcohol.

The Desert Locust Survey maintained close co-operation with the Anti-Locust Research Centre and extra officers were recruited to deal with the re-invasion of the Somali Peninsula. Promising results were obtained in initial field aerial spraying tests with diazinon, and aerodel (11 per cent gamma-BHC) proved ideally suited for spraying locusts even in bad weather over difficult terrain. By the time the locusts started to breed in October 1957 the Survey had concentrated 30 officers and 125 vehicles in the Somaliland Protectorate and eastern Ethiopia and provided 65 vehicles in Somali with adequate supplies of bait and insecticides. Concern is expressed at the almost continuous nature of the threat since the War, but reference is also made to the development of international co-operation under the Food and Agriculture Organization of the United Nations and elsewhere.

SOIL ZOOLOGY

A COLLOQUIUM on "Research Methods in Soil Zoology", organized by the Soil Zoology Committee of the International Society of Soil Science, was held at Rothamsted Experimental Station during July 10–14, by permission of the director (Sir William Ogg) and the director of the Department of Entomology (Dr. K. Mellanby) and with financial assistance from Unesco and the Shell Petroleum Co., Ltd.

Although the titles of the 95 papers give an impression of great diversity, it is possible to distinguish certain themes which emerged from the papers and from the discussions and which are worth reporting to a wider public. This is so largely because the papers, grouped into five sections, were made available to members beforehand and (with a few exceptions) were not read at the conference; instead, the section recorders provided reviews of the papers, and these were then followed by really useful discussions, during which a reasonable amount of time was available for consideration of the most worthwhile topics.

Perhaps the most constantly recurrent theme was one which belongs most appropriately to Rothamsted, namely, the nature of the relationship between the biologist and the mathematician; this was introduced in the first section on "Methods of Sampling and Analysis". As J. G. Skellam pointed out, the relationships between the structures of the two subjects are indirect, and it is not always appreciated that it is the biologist and not the mathematician who must provide the working hypotheses that are to be tested by the application, for example, of statistical methods to his data. If the biologist simply provides a mass of undigested figures the mathematician is scarcely to be blamed if he erects his system of tests on a mathematically convenient but biologically unrealistic set of hypotheses. This theme was amply illustrated by speakers on such diverse topics as the spatial distribution of soil fauna, the analysis of species composition of communities and the measurement of efficiency of extraction methods.

A variant of this theme, on which the views of speakers proved irreconcilable, was the roles of experiment and statistical analysis in biological science. Whereas Dr. K. Mellanby and Prof. Kühnelt (Vienna) were advocates of the elegant laboratory experiment resulting in the production of few, but reliable, data and the support of field work wherever possible by laboratory experiment, Dr. J. E. Satchell (Merlewood) and others were strongly in favour of the use of mathematical analysis on selected field data, and Prof. M. Ghilarov (Moscow) and Prof. Franz (Vienna) were of the opinion that such methods are usually inappropriate owing to imperfect sampling methods and patchy distribution of soil organisms. Although there must obviously be room for great differences in approach to so wide a field, it was evident that the use of statistics as an analytical tool is still rather limited outside the English- and French-speaking countries and that the need for the kind of work that we associate with the name of Rothamsted is still great.

A most useful contribution to this liaison work was the first paper by M. J. R. Healy, whose survey of basic statistical techniques concluded with sections on non-random distributions and the use of transformations in variance analysis. Prof. H. R. Debauche (Louvain) as recorder of this section reviewed a wide range of topics in which mathematical techniques are appropriate to ecological studies, including, especially, the analysis of production and energy flow and the delimitation of communities. J. G. Skellam introduced a mathematical analysis of the use of extraction methods for population estimation with emphasis on the effect of extraction-rate and population heterogeneity on accuracy. Also from the Nature Conservancy D. D. Mountford introduced a new method for classifying species records into associated groups; this method was devised for the analysis of records obtained by J. E. Satchell covering between 50 and 100 species at each of ten sites, and is noteworthy, first, because an estimate of statistical significance is provided, and secondly, because this was the first occasion at the Colloquium on which the use of an electronic computer was reported, the distribution data for the species having been fed to the machine at Rothamsted on punch cards. Evidently this is but the beginning of a further field of application for these machines, for the next paper, by Dr. R. E. Blackith (Imperial College of Science and Technology), acknowledged their value in a discussion of the fields of application of multivariate analysis. Further contributions to this section were concerned with the study of distributions of aggregated populations along more or less familiar lines, Dr. R. D. Hughes (Wellesbourne) introducing a novel technique for estimating mean radius and frequency of patches of high density.

The second section, on "Extraction Methods", was reviewed by the recorder, Dr. P. W. Murphy, and was followed by demonstrations of apparatus and techniques. Some of the methods were already familiar, but new features introduced in the twenty-four papers or in the discussion included an extension of the use of suction methods for collecting ground surface arthropods, a machine for brushing microscopic species from leaves, the use of marking tech-

niques both for population estimation and for assessment of the accuracy of extraction methods, the improvement of the extraction efficiency of Tullgren funnel methods by the control of humidity and the use of solar heat for funnels in large-scale faunal survey work in the U.S.S.R. Reports of comparative trials of different methods served to underline the variations in efficiency which occur when the same method is used on different animal groups, on different soil types and even in the hands of different operators.

The third section, on "Systematics and the Ecologist", was summarized by Dr. H. Gisin (president of the Committee), who was especially concerned with the relationships between the ecologist and the taxonomist when co-operating in the study of the more 'difficult' groups such as the mites and the Collembola. The taxonomist has the right to receive material in good condition, to be spared preliminary sorting and to make decisions about publication on taxonomic matters, in exchange for which the ecologist can expect rapid determination of selected material and the provision of keys at least to generic level. When determinations to species are required, a fruitful field of co-operation is provided because large ecological differences may accompany only small variations in morphology. There were differences of opinion about the value of reference collections, at least when these are uncritically used, and about the extent to which keys should be carried to the level of species, and should be limited to single countries or natural faunistic regions. Prof. Franz announced a scheme for the uniform publication of a series of keys to the European soil fauna under his editorship and published in Berlin, and Dr. G. O. Evans (British Museum, Natural History) described another scheme for a European key to genera of mites in collaboration with Dr. J. Balogh (Budapest) and Dr. K. Strenzke (Wilhelmshaven). A number of taxonomists present made pleas for correct mounting and preservation methods (not all of which were compatible) and described suitable techniques.

The fourth section, on "Soil Zoology and Pedology", was introduced by Prof. H. Franz (Vienna), who insisted on the need for close liaison between the two subjects. The influence of the past history of a soil on its present fauna and characteristics and the nature of the distinction between the aquatic and aerial components of the soil fauna were discussed. The papers in this section included two methods for studying the nature and rate of decomposition of the latter.

The fifth section, on "Laboratory Techniques", was ably reviewed by J. d'Aguilar (Versailles) and included twenty-nine papers on three main fields, namely: (1) the study of soil by sectioning, microscopical and photographic techniques; (2) the use of numerous special culture methods for different groups of soil animals; and (3) methods for studying metabolism of soil animals by means of radioisotopes and of a special micro-respirometer. Further details of techniques were provided in the discussion together with some sobering examples of ways in which unforeseen factors can cause faulty experimental results; in this way the complementary roles of laboratory and field work were vividly illustrated—at least to those not too far committed to one or other school.

The value of the colloquium was much enhanced by the efficient organization of the secretaries, Dr. P. W. Murphy (Rothamsted) and J. d'Aguilar (Versailles), and owed much to the wise and sympathetic chairmanship of Dr. Mellanby and his colleagues. The discussions throughout were recorded, and translation was ably supplied between English and German. The proceedings, including the discussions, are to be published this year by Messrs. Butterworths in two volumes, the first covering Sections 1–4, and the second containing the papers in Section 5 which are thought likely to have a wider appeal.

A. MACFADYEN

BRITISH COAL UTILIZATION RESEARCH ASSOCIATION

THE annual report* of the British Coal Utilization Research Association for 1957 includes a brief review of the Association's research work. The major objectives during the year were to help in bridging the gap between suitable coal supplies and energy requirements and assisting the implementation of the Clean Air Act. The development of a small-pipe system for central heating was substantially completed, as was work on the formulation of procedures and the design of equipment for measuring the emission of grit and dust; an automatic particle counter and sizer developed by the Association is now in commercial production. Tests at Leatherhead on the stationary locomotive boiler were also completed and further experimental work with the model furnace-tube plant has reduced the unaccounted heat loss to about 2–3 per cent Increased attention is now being given to automatic control of firing appliances and, with the increasing use of small coal in such equipment, to the study of grit and dust catchers. An improved method was devised for controlling the air distribution through the fuel beds of chain grate stokers for boilers of the shell type, and methods of alleviating the rapid corrosion at the cold end of a boiler, particularly of air heater surfaces, have also been investigated.

Laboratory studies continued on the mode of release of chlorine, alkalis and sulphur from coal up to 1,500° under shock heating conditions, and further tests have shown that the potential tar yield of the coal as defined by the Gray King Assay provides a good basis for comparing coals for tar content. Problems associated with the production of fuel or synthetic gas in gasifiers operating at high pressures and temperatures, at which the ash is in the form of liquid slag, were also investigated with the view of utilizing small low-rank coal.

Basic research included investigations into the chemical basis of the plastic softening of coals, with the view of better control of coking properties; studies of the fundamental reactivity of graphite towards oxidizing gases in relation to porous structure; and on the solvent extraction of a medium-rank coal with

* British Coal Utilization Research Association. Annual Report 1957. Pp. ii+62+4 plates. (Leatherhead: British Coal Utilization Research Association, 1958.) 10s.

tar oils, using ultrasonic extraction equipment, to produce a smokeless fuel with coal extract as a by-product. A further step was taken towards the establishment of the absolute significance of measurements of surface areas of coals and carbonized coals by the heat of wetting method. A list of reports and published papers is appended.

JUBILEE OF ESKDALEMUIR OBSERVATORY

ESKDALEMUIR Observatory, the geomagnetic and meteorological observatory in a remote situation in north-east Dumfriesshire, was founded on May 11, 1908. It was established because the westward extension of electric tramways from London rendered the magnetic observations at Kew Observatory insufficiently exact for many purposes. Compensation of £10,000 was paid by the tramway company to the National Physical Laboratory, under which the geomagnetic work at Kew Observatory then came, and this money was used to build Eskdalemuir Observatory. The site is reputed to have been chosen by placing a coin on a map so as not to intersect any town or railways within 10 miles. Eskdalemuir came with Kew under the control of the Meteorological Office in 1910.

The history of geophysical work at Eskdalemuir and plans for the future are given by the present superintendent, Mr. M. J. Blackwell, in the May 1958 number of the *Meteorological Magazine*.

The Observatory has been throughout a first-order geomagnetic and meteorological observatory. Until 1925, in which year the seismographs were transferred to Kew, it was also a seismological observatory.

Three sets of magnetic instruments, each set recording horizontal force, declination and vertical force, are now in use. The three sets are of different sensitivity and traversing rate and serve different purposes. The magnetographs are in a non-magnetic underground house, the temperature of which is thermostatically controlled. Absolute observations of the magnetic elements are also made, of course. It is interesting to note that the declination of 18° W. in 1908 has now decreased to less than 11° W. The records of the magnetographs are analysed at the Observatory, work which continually becomes more complex.

In meteorology and atmospheric physics, extensive work is done in climatology, solar radiation, atmospheric ozone and atmospheric electricity, to mention but a few of the main activities. The apparatus for measuring precipitation includes a variety of standard and experimental rain and snow gauges. Special observations undertaken during the present International Geophysical Year are chemical sampling of air and rain-water, measurements of evaporation and investigation of the vertical distribution of ozone.

The situation of the Observatory has meant that accommodation has to be provided for all the staff, with associated domestic workers, who form a small community—in early years a very isolated one.

Mr. J. Crichton, a former superintendent of Eskdalemuir, wrote in the *Meteorological Magazine* in 1950: "So far no encroachment by artificial (magnetic) disturbance has taken place". In expressing good wishes to the Observatory for its next fifty years, we can hope that the happy state of absence of artificial disturbance will continue.

FORESTRY POLICY IN BRITAIN

THE Minister of Agriculture, Fisheries and Food, Mr. J. Hare, in a statement on forestry policy in the House of Commons on July 24, said that the Government had reviewed the position and decided to fix the planting programmes of the Forestry Commission for periods of ten years at a time. For 1959–63 the programme would be about 300,000 acres, and for 1964–68 it would be reduced to about 235,000, when the Commission's existing plantations began to come into full production. The size of subsequent programmes would be reviewed in five years time in the light of the national needs, and in deciding when planting should take place, special attention would be paid to the upland areas, particularly in Scotland and Wales, where expansion of forestry would provide needed diversification of employment and important social benefits.

The Government proposed to continue its support to private woodland owners, particularly through the dedication scheme, but the present maintenance grant of 5*s*. 6*d*. per acre would be replaced by a management grant of 18*s*. per acre on the first 100 acres, 12*s*. on the next 100 and 7*s*. per acre on the rest. The planting grant would be continued, but for approved woodlands would be raised from one half to the full rate per acre. These increased grants are contingent on the formation of an effective woodland owners' association, as recommended by the Watson Committee. The grants for thinning and poplar planting would be terminated, and, in reviewing in future years the level of grants, trends in receipts for private woodlands as a whole as well as trends in costs would be considered. Eventually—in twenty to thirty years time—the level of assistance needed by way of planting and management grants should become nominal. The system of felling licences would be continued; a statutory instrument would eliminate the need for a licence for fellings in dedicated woodlands, and in general licences would be freely granted for other fellings, subject to the existing arrangements for consultation with planning authorities. Normally, licences would be conditional upon restocking. In reply to questions, Mr. Hare said that the Forestry Commission would consult the woodland owners' organizations in assessing trends in receipts and costs, that his Department was working in the closest possible co-operation with the Secretary of State for Scotland and the Forestry Commission and that there would be no interference with the assistance now given by the Commission to farmers who sought to replant scrub land and land covered with bushes and trees.

LEAF AREA AND PASTURE GROWTH

AGRICULTURISTS are ultimately concerned with yield per unit area of land, and it is the aim of plant physiologists interested in agricultural problems to attempt to analyse the causes of variation in yield. In a recent review entitled "The Significance of Leaf Area in Pasture Growth", by C. M. Donald and J. N. Black of the Waite Agricultural Research Institute, Australia (*Herbage Abstracts*, 28 ; 1958), it is pointed out that of the chief physical factors limiting yield, water and nutrients have received more attention than light, particularly in pasture studies. Under field conditions the amount of light falling on a pasture is not controlled by man, nor is it subject in any particular locality to the same degree of annual fluctuation as is rainfall. When light is the only limiting factor the ultimate capacity of a species to produce dry matter depends on the degree to which a community of such plants can exploit the light falling on it. This will depend on the habit of the plant. The yield, therefore, will depend to a great extent on the area of photosynthetic tissue which is able to intercept light energy, that is, the leaf area. Consequently, the important factor is the relationships of leaf surface to soil surface, and this was first put on a quantitative basis by D. J. Watson in his concept of 'leaf area index', which is defined as the leaf area per unit area of land. This index has the virtue of being a measure of the whole crop rather than of individual plants.

Watson postulated that the greatest dry-matter production would occur when the maximum leaf area coincided with seasonal conditions most favourable for photosynthesis. The development of a leaf system capable of intercepting a high proportion of the incoming light energy is of great importance in the growth of field crops ; at values of the index below this level, maximum growth cannot occur. On the other hand, at higher values mutual shading of leaves may result in a reduction in growth-rate of the crop.

The limited number of determinations of the leaf area index of pastures suggests that these values may considerably exceed that of crops, and it is suggested that there may be several reasons for this. First of all, the types of plants which have been selected by man to sow pastures for his animals are those with heavy leaf production. A second major aspect affecting the index is the greater number of plants per unit area in pastures compared with crops.

The authors emphasize that the application of the concept of leaf area index to problems of pasture growth is of great potential value. They stress that provided other conditions are optimum light is the only limiting factor determining productivity of a pasture and that efforts to increase production beyond the high levels already recorded in some environments may be in vain unless this limiting factor is fully appreciated. With these concepts clearly in mind the object in pasture management will be to pass as rapidly as possible to the optimum leaf area index for pasture growth and then to hold the pasture at this level. Any operations which delay this process, such as early grazing when the index is low, will substantially delay attainment of the optimum and may have an effect on subsequent production quite disproportionate to the amount removed. Other aspects of pasture production capable of analysis in terms of leaf area index are pointed out.

LABORATORIES IN GRAMMAR SCHOOLS

THE inadequate provisions for science teaching in grammar schools have been brought out only too clearly in the answers to a questionnaire which was sent to some 2,000 schools about a year ago*. The 472 schools from which data were considered are widely distributed over the whole of England and Wales, with a slight bias towards London and the surrounding counties.

These schools contained about 25,000 pupils, of whom 90·9 per cent took some science. This is a reassuringly high proportion which does not vary much between the extremes of 86·5 per cent in independent boys' schools and 93·9 per cent in the small grammar schools. How much science was studied was not indicated, and, for many pupils, the time devoted to science may be very small, perhaps only one or two periods a week.

Almost all pupils do some science in the first three years of their secondary courses. In the fourth year the percentage figures drop a little, and in the fifth (O-level) year, about 90 per cent of pupils are taking science, although not all of these will offer science subjects in the O-level examination. The dropping of science in the fourth and fifth years may occur because subject specialization is already starting, or it may be because sufficient teachers or laboratories or both are not available.

In the sixth form there is a considerable fall in the percentage of pupils taking some science. This is particularly marked in the girls' schools, being only 47 per cent in the two categories of grammar schools having 300–399 pupils and 400–549 pupils, 44 per cent in the 500–699 category, and 39 per cent in the larger schools. The corresponding figures for boys' schools are 66, 60, 61 and 65 per cent.

Expressed as a percentage of total sixth-form numbers, the figures for girls' schools are about half of those for boys' schools. Since the sixth forms in girls' schools are smaller than in boys' schools, the number of girls specializing in science is less than half the number of boys.

These results support the view that girls' schools could be an important source of the increased number of scientists required in industry, in Government departments, for teaching and for many other purposes.

One question required each school to state its total number of laboratories and other rooms having a special connexion with the teaching of science. Using

* *School Science Review*, **39**, No. 139 ; June 1958.

the standards framed by other responsible bodies, namely, the Ministry of Education and the Committee of the Industrial Fund for the Advancement of Scientific Education in Schools, the figures reveal the inadequacy of existing provisions.

More than half of the schools which appear to be adequately provided with laboratories by Ministry of Education standards are girls' and mixed schools which, if considered by the higher standards the Ministry adopts for boys' schools, would not be regarded as adequately provided.

An attempt was also made to discuss the existing provision for laboratory assistance. Out of 362 maintained grammar schools answering the questionnaire, 92 (25 per cent) have no laboratory assistance at all. 31 (8·6 per cent) have only part-time help, and 78 (22 per cent) have only one junior assistant. 85 per cent are not adequately provided with laboratory assistance.

The amount spent each year on science in grammar schools is revealing. 144 out of 337 maintained grammar schools, that is, 43 per cent, had less than 8*s*. per year per pupil taking science. At one extreme an independent boys' school has £3 10*s*. per year for each pupil taking science, while one direct-grant boys' school has £2, and one independent girls' school has £1 15*s*. 9*d*. The highest figure shown by a maintained boys' school is £1 8*s*. 8*d*., and for girls' £1 5*s*. 10*d*. At the other extreme, one boys' grammar school has only between 1*s*. 3*d* and 3*s*. 6*d*. annually for each pupil taking science. Financially, as in other respects, girls' schools are, in general, considerably worse off than boys' schools. It appears that the schools with less than 10*s*. yearly for each pupil taking science (that is, about half of the grammar schools) are being subjected to conditions which are frustrating and inimical to the work they are called upon to do.

Schools were invited to make general comments concerning difficulties encountered in science departments. 256 schools gave seventy different reasons for the difficulties they faced. Among reasons given which received considerable support were: (*a*) accommodation generally cramped and crowded (79); (*b*) sharing accommodation or using another school's science laboratories (13); (*c*) laboratories too small (14); (*d*) no advanced laboratories (16); (*e*) laboratories widely dispersed—in several cases by a mile or more (19); (*f*) no preparation rooms (10); (*g*) out-of-date and/or ill-designed laboratories (14); (*h*) laboratories used as form rooms (21); (*i*) much science teaching in class-rooms (16); (*j*) serious shortage of storage space (22); (*k*) inadequate finance, lack of capital grant, etc. (33); (*l*) shortage of staff (28); (*m*) lack of suitable qualified staff (28); (*n*) too many young and inexperienced staff (14); (*o*) staffing ration not generous enough, time-table too heavy, etc. (17); (*p*) classes far too large (24); (*q*) no laboratory assistants (22); (*r*) inadequate or poor quality laboratory assistants (48).

MEDICAL RESEARCH IN INDIA

THE Technical Report of the Scientific Advisory Board, published annually by the Indian Council of Medical Research (pp. xii+290. New Delhi: Indian Council of Medical Research, 1957. Rs. 1), has primarily included detailed reports of work done under all the research schemes financed by the Council during the year. The report for 1956, as a measure of economy, contains only short abstracts of work done under research schemes financed during 1955–56. Of these, 52 relate to chemical research, 5 each to cholera and leprosy, 9 to malaria and other arthropod-borne diseases, 10 to tuberculosis, 3 to venereal diseases, 4 each to environmental hygiene and sanitation and to industrial health, 16 each to maternal and child health, to nutrition, to physiology and pharmacology, 1 to mental health, and 7 to virus diseases. Besides details of the Scientific Advisory Board and its Advisory Committees and Sub-committees, the report includes lists of papers published by workers of the Indian Council of Medical Research in 1956 and of those dealing with work done under the auspices of the Council and published in 1956.

From this array of papers it is almost impossible to select any for particular mention, but as of more general scientific interest reference may be made to the electron microscopic investigations on micro-organisms under Prof. K. N. Das Gupta at the Institute of Nuclear Physics, Calcutta; to the leprosy research work under Dr. N. Mukerjee at the School of Tropical Medicine, Calcutta; the laboratory and field investigations at the Malaria Institute of India, Delhi, on resistance in houseflies and mosquitoes to chlorinated hydrocarbon insecticides and the comparative efficiency of different insecticides against mosquitoes; on the control of *W. malayi* filariasis in Travancore–Cochin with diethylcarbamazine; the studies in vitamins, proteins and fats at the Nutrition Research Laboratories, Coonoor; and on the biosynthesis of ascorbic acid by the Nutrition Research Unit at the University College of Science and Technology, Calcutta.

The Indian Council of Medical Research has also issued a review of the progress made in the research projects it sponsors covering the whole of the period 1950–57 (pp. v+169. New Delhi: Indian Council of Medical Research, 1957). The survey is intended to convey some idea of the philosophy and approach of the Indian Council of Medical Research to the development of medical research in India, the contribution being made towards public health and the direction in which it is moving. Among the features stressed in the introductory review are the measures taken against malaria, filariasis, leprosy, plague, tuberculosis, trachoma and preventable blindness; the new outlook on cholera and venereal disease and malnutrition and virus disease. The survey gives a concise and readable account of the organization of medical research in India, both in diverse fields of research, between different institutions and of the balance between fundamental and applied research. Besides this section, which displays a vision, determination and resource on which the Council and Government of India are to be highly congratulated, there is a section on progress in selected fields, while a further section on the year ahead, as well as the concluding section, show that the Council is fully alive to the factors upon which effective scientific work depends and is seeking to provide the scientist, not simply with the institutions and equipment, but also with the conditions of service in which he can give of his best.

PARTITION OF PROTEINS IN LIQUID POLYMER–POLYMER TWO-PHASE SYSTEMS

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THE behaviour of proteins in liquid two-phase systems has been of increasing interest because partition chromatography and counter-current extraction have been shown to be useful tools for the fractionation of protein mixtures, thus complementing adsorption chromatography and zone electrophoresis[1,2].

Suitable two-phase systems for proteins are difficult to construct, mainly because (*a*) most proteins are easily denatured or precipitated by the organic solvents usually used for preparing phase systems, and (*b*) proteins are macromolecules and have, therefore, a general tendency to concentrate only in one phase, that is, one obtains either very high or very low partition coefficients. When, as sometimes happens, two proteins concentrate in different phases this is, of course, an advantage since it leads to large separation factors. If, however, two proteins go to the same phase it is difficult to apply partition chromatography or counter-current extraction for their separation.

To overcome these difficulties, phase systems have been used which are made by mixing water, salt, and organic solvents soluble in water, like alcohol, glycol ethers, etc.[1–7], in such proportions that the composition is near the plait point. Such systems dissolve many proteins and give finite partition coefficients. Near the plait point the partition coefficient is, however, extremely sensitive to small changes in the composition of the phase system which, in turn, is sensitive to variations in the temperature. The composition and temperature must therefore be controlled very carefully.

This communication describes some results that have been obtained when proteins are distributed between two-phase systems composed of water and two high polymers soluble in water. Such systems have been used for the fractionation of cellular particles[8]. Ref. 8 also contains references to the theory and experiments on polymer–polymer phase separation in general. It is well known that when two different polymer solutions are mixed, phase separation occurs, even at low concentrations of the polymers. Of the two phases thus obtained, one contains almost all of one polymer and the other phase almost all of the other. Both phases, however, usually contain between 80 and 99 per cent of water, depending upon the type of polymer used and its mean molecular weight.

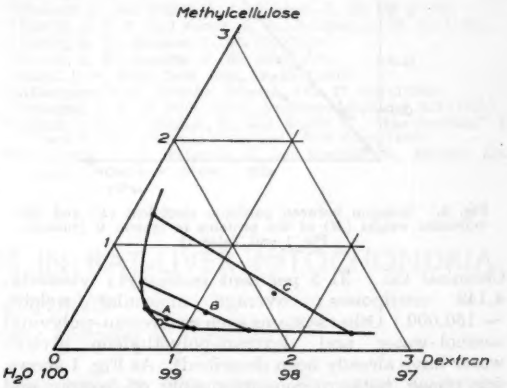

Fig. 1. Part of a phase diagram with tielines for the system dextran–methylcellulose–water at 20° C. O, plait point. The compositions of *A*, *B* and *C* have been used for the determination of the partition coefficients of proteins (see Table 1). (Composition per cent w/w.)

Fig. 1 shows a typical triangular phase diagram of a polymer–polymer system, dextran, methylcellulose and water. (The dextran was obtained from AB Pharmacia, Uppsala: intrinsic viscosity, 0·68; weight average molecular weight, 1,800,000; number average molecular weight, 280,000. The methylcellulose, 'Methocel', was obtained from the Dow

Table 1. The Partition Coefficient of certain Proteins in the Two-phase System Dextran–Methylcellulose–Water at the following three total Compositions (w/w) (compare Fig. 1):
A, 0·80 per cent dextran, 0·30 per cent methylcellulose, 98·90 per cent buffer + protein; *B*, 1·10 per cent dextran, 0·36 per cent methylcellulose, 98·54 per cent buffer + protein; *C*, 1·58 per cent dextran, 0·54 per cent methylcellulose, 97·88 per cent buffer + protein
Protein content in the total system 0·1–0·2 per cent w/w

Protein	Mol. weight $\times 10^3$ (ref. 10)	Partition coefficients			*p*H	Molar concentration of phosphate buffer	Kind of protein determination†
		System *G*	System *B*	System *A*			
Ribonuclease	13	0·96			6·8	0·01	*N*
Cytochrome *c*	16	0·95			6·0	0·01	E_{410}
Pepsin	36	1·4			4·6	0·01	*N*
Ovalbumin	44	0·82			6·0	0·01	*N*
Laccase (fungal)	*	0·8			6·0	0·01	*EA*
Hæmoglobin (man)	67	0·79	0·96	0·99	6·0	0·01	E_{420}
Serum albumin (man)	69	0·81			6·0	0·01	*N*
Enolase	68	0·76			6·0	0·01	*EA*
Caeruloplasmin	151	0·7			6·0	0·01	*EA*
Gammaglobulin (man)	156	0·6			6·0	0·1	*N*
Fumarase	204	0·65			7·0	0·01	*EA*
Catalase	250	0·75			6·0	0·01	E_{405}
C-Phycocyan	270	0·63			6·0	0·01	E_{620}
R-Phycoerythrin	290	0·68	0·85	0·95	6·0	0·01	E_{495}
Fibrinogen (man)	340	0·3			6·0	0·1	*N*
Hæmocyanin eighth (*Helix pomatia*)	1,000	0·25	0·5	0·8	8·5	0·01	*N*
Hæmocyanin whole (*Helix pomatia*)	9,000	< 0·1	—	0·5	6·0	0·01	*N*
Tobacco mosaic virus	40,000	—	—	0·06	6·0	0·01	*N*

* Molecular weight not determined. $S_{20} = 6·0$ (ref. 11). A molecular weight of 60,000 was assumed when plotting the data for laccase in Fig. 2.
† *N*, nitrogen determination according to Kjeldahl; *EA*, enzyme activity; *E*, extinction. Figures indicate wave-length in mμ.

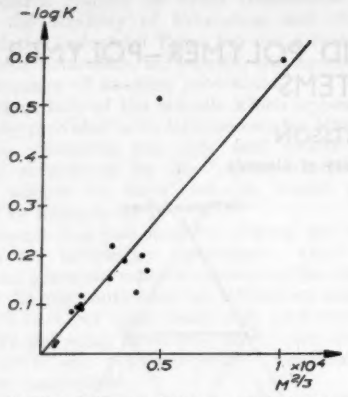

Fig. 2. Relation between partition coefficient (K) and the molecular weight (M) of the proteins in system G (compare Fig. 1 and Table 1)

Chemical Co.: 31·5 per cent methoxyl; viscosity, 4,149 centipoises; average molecular weight, ~ 150,000.) Other systems such as dextran–polyvinyl alcohol–water and dextran–polyethylene glycol–water have already been described[5]. As Fig. 1 shows, one phase (bottom) consists mainly of dextran and water, and the other (top) of methylcellulose and water, except very near the plait point.

Three compositions, represented by the points A, B, C on the phase diagram (Fig. 1), were selected for further study.

The partition coefficient of a number of proteins was determined in these systems in the following way. One gram of an aqueous protein solution previously dialysed against buffer (see Table 1 for concentrations) was added to 9 gm. of a mixture of dextran, methylcellulose and buffer in a separating funnel so that the desired composition as indicated in Table 1 was obtained. The mixture was allowed to equilibrate in a thermostat at 20° C. and was shaken by turning the funnel upside down about fifty times. The phases were then allowed to separate overnight. Portions of the top phase and the bottom phase were then collected, weighed and diluted appropriately for the determination of the protein content.

As indicated by the results in Table 1, all proteins, except pepsin, concentrate in the dextran-rich phase.

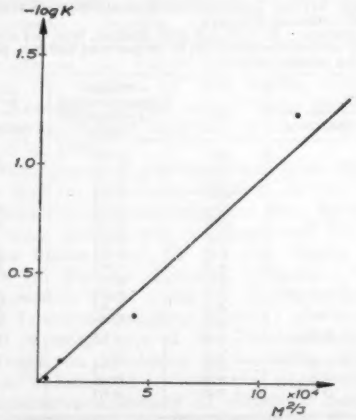

Fig. 3. The same as in Fig. 2 in system A

Even far from the plait point most of the proteins have measurable finite partition coefficients between 0·1 and 1. This differs considerably from the results obtained with low molecular weight phase systems[4–7].

There is a significant correlation between the partition coefficients and the molecular weights of the proteins (Table 1). The conditions were not exactly similar for all the proteins, so the partition coefficients cannot strictly be compared, but the general tendency is obvious. The pH and ionic strength also affect the partition coefficient, but not to the same extent as the molecular weight, provided the protein molecule is stable. If the protein molecules aggregate, or dissociate, due to changes in pH or ionic strength, these latter factors will, however, affect the partition coefficient considerably.

The results in Table 1 (see also Figs. 2 and 3) are compatible with the theory of the partition of macromolecules in two-phase systems originally worked out by Brönsted[8]. He derived the following relationship between partition coefficient (K) and the molecular weight (M) of 'isochemical' substances:

$$\log K = \frac{\lambda}{RT} \cdot M$$

where λ is a constant characteristic of the phase system and the type of substance. If the partitioned molecules are globular, like most proteins, M should be replaced[9] by $M^{2/3}$.

A closer comparison with theory will be postponed until more experimental results are available and a wider range of partition coefficients have been determined.

It seems that analysis of the partition in this phase system would be a simple way to obtain at least a rough estimate of the molecular weights of proteins. For example, the determination of the partition of enzymatic and hormonal activity and substances of low molecular weight in crude mixtures like cell homogenates could give information as to the size of the particles to which these activities and substances are attached.

The proteins seem to dissolve without denaturation. The enzymes retain at least 90 per cent of their activity. With hæmocyanin, however, precipitation was occasionally observed at low pH values.

The system dextran–methylcellulose–water is comparatively insensitive to changes in temperature. The time necessary for the two phases to separate is about 12, 6 and 1 hr. for the systems A, B and C, respectively. The top phase of system G is rather viscous, and small drops of the bottom phase remain in it for some time, but this introduces no serious error in the determination of partition coefficients between 0·1 and 1. In both systems, B and C, the two phases can be separated in a few minutes by centrifugation at low speed, for example, 2,000 g, without taking special precautions to control the temperature.

Table 2. Partition of Proteins in the System Dextran–Polyethylene Glycol–Water with different Fractions of the Polymers

The proteins are partitioned with finite partition coefficients but in favour of the phase indicated in the columns under the proteins. Compare ref. 8, Table 4. *PEG*, polyethylene glycol

Phase system. (Nos. indicate mol. wt. of polymers)	Hæmoglobin	Serum-albumin	Phycoerytrin	Phycocyan
Dextran 20,000–*PEG* 6,000–7,500	dextran	dextran	dextran	dextran
Dextran 1,800,000–*PEG* 6,000–7,500	,,	,,	*PEG*	*PEG*

Other polymer systems are also able to dissolve proteins. In the system dextran–polyethyleneglycol–water, the distribution of some proteins is highly dependent upon the molecular weight of the two polymers. Thus, the partition coefficient can be shifted from less than one to greater than one by replacing one of the polymers by another fraction of the same polymer with a different molecular weight (Table 2). This is analogous to the distribution of particles in similar phase systems as described earlier[8].

An obvious difficulty of this method of protein separation is that the phase system components cannot easily be removed by dialysis. However, this could possibly be overcome by zone electrophoresis or adsorption of the proteins, on an ion exchanger or calcium phosphate, for example.

Further work is in progress, and a detailed report will probably be published later.

I wish to express my thanks to Prof. A. Tiselius for stimulating interest and support. The work has been aided by grants from the State Council of Technical Research.

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FORMS OF PYRIDINE NUCLEOTIDES IN RAT-LIVER MITOCHONDRIA

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In connexion with studies to determine whether mitochondrial isocitric dehydrogenase is a linked nucleotide with di- or tri-phosphopyridine, it was necessary completely to deplete the mitochondria of their pyridine nucleotides. The depletion was followed by determining the amounts of oxidized and reduced di- and tri-phosphopyridine nucleotides by specific enzymic, fluorometric methods[1] applied to acid and alkali extracts of mitochondria after different times of incubation. Fig. 1 describes an experiment in which the mitochondria were incubated with 40 mM phosphate (Fig. 1 A) and with 1·9 mM adenosine diphosphate + 33 mM nicotinamide (Fig. 1 B). Fig. 1 A shows that the depletion of diphosphopyridine nucleotide consists of two phases: first, the conversion of reduced diphosphopyridine nucleotide to its oxidized form; and secondly, destruction of the oxidized diphosphopyridine nucleotide. The oxidation of reduced triphosphopyridine nucleotide occurred somewhat slower but in a similar manner. Nicotinamide inhibited the destruction of the oxidized nucleotides (Fig. 1 B).

Fig. 1 also shows that during incubation with 40 mM phosphate or 1·9 mM adenosine diphosphate the content of diphosphopyridine nucleotide reacting with yeast alcohol dehydrogenase increased to a value above the total of both oxidized and reduced

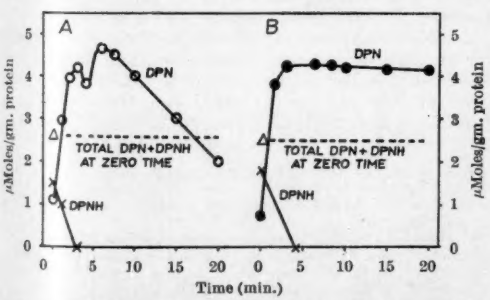

Fig. 1. The mitochondria were incubated at 30° C. with 40 mM phosphate (A) and with 1·9 mM adenosine diphosphate + 33 mM nicotinamide (B)

nucleotides at zero time. The extra diphosphopyridine nucleotide must be derived from some precursor. Since the increase occurred after incubation with phosphate, adenosine diphosphate or 2,4-dinitrophenol but not with adenosine triphosphate, it is probably due to the breakdown of a compound containing diphosphopyridine nucleotide rather than to a synthesis of that nucleotide. Moreover, pyridine nucleotide synthesis is localized in the nucleus[2]. A similar increase in the total of oxidized and reduced triphosphopyridine nucleotides during the incubation indicates that the increased diphosphopyridine nucleotide does not result from a conversion of triphosphopyridine to diphosphopyridine nucleotide. The fact that the compound containing diphosphopyridine nucleotide yields this nucleotide on addition of phosphate, adenosine diphosphate or 2,4-dinitrophenol but not adenosine triphosphate strongly suggests that it is connected with oxidative phosphorylation. In fact, it has the properties expected of a diphosphopyridine nucleotide compound (DPN $\sim I$) which has been postulated as an energy-rich intermediate of oxidative phosphorylation[3,4]. The concentrations of phosphate, adenosine diphosphate or 2,4-dinitrophenol necessary to give a half-maximal appearance of diphosphopyridine nucleotide from its reduced form and the energy-rich intermediate were 3·5 mM, 185 μM and 4·5 μM, respectively. The half-maximal concentration of adenosine diphosphate in the presence of 2 mM phosphate was 70 μM. These K_m values follow the same order as in oxidative phosphorylation.

The amounts of the various forms of the pyridine nucleotides in mitochondria are given in Table 1. Recovery experiments with added reduced di- and tri-phosphopyridine nucleotides ranged from 90 to 110 per cent. Thus the increase in total pyridine nucleotide after incubation is not due to an under-estimation of reduced di- and tri-phosphopyridine nucleotides.

Eisenhardt and Schrachinger[5] have estimated the amount of high-energy compounds present in liver mitochondria, non-respiring owing to lack of phosphate acceptor, by the rapid increase in the concentration of adenosine triphosphate which occurs

Table 1. Concentrations of different forms of Pyridine Nucleotides in Rat-Liver Mitochondria

The pyridine nucleotides were determined at zero time and after 7 min. at 30° in the presence of 40 m*M* potassium phosphate or 1·9 m*M* adenosine diphosphate. The values, expressed as μmoles/gm. protein, are the means from 13 experiments

	0 min.	7 min.
DPN	1·05	4·32
DPNH	1·6	0
DPN + DPNH	2·65	4·32
DPN ~ *I*	1·67	—
TPN	0·27	4·85
TPNH	2·1	0
TPN + TPNH	2·37	4·85
TPN ~ *I*	2·48	—

immediately after adding adenosine diphosphate. This value, 1·5 μmoles/gm. protein, is similar to our value for the diphosphopyridine nucleotide intermediate (1·67 μmoles/gm. protein).

Amytal (5-amino-5-ethylbarbituric acid), in a concentration of 1·8 m*M*, completely blocks oxidations linked with diphosphopyridine nucleotide in rat-liver mitochondria[6], acting between flavoprotein and diphosphopyridine nucleotide[7]. In the presence of 0·8 m*M* amytal, 33 m*M* nicotinamide and 1·9 m*M* adenosine diphosphate, the reduced diphosphopyridine nucleotide content of liver mitochondria decreased slowly, while the content of the oxidized form remained relatively constant. Under these conditions 75 per cent of the reduced diphosphopyridine nucleotide disappeared, presumably by conversion to the disphosphopyridine nucleotide intermediate. The diphosphopyridine nucleotide which disappeared could be recovered by dilution and addition of 2,4-dinitrophenol. A higher concentration of amytal (1·8 m*M*) completely inhibited any change in the concentrations of diphosphopyridine nucleotide or its reduced form.

Alterations in the disphosphopyridine nucleotide intermediate concentration can also be produced by the addition of substrate. The addition of β-hydroxybutyrate to liver mitochondria at 0° C., in the presence of 3·0 m*M* magnesium but in the absence of phosphate acceptor, caused the concentrations of both oxidized diphosphopyridine nucleotide and its reduced form to increase in 90 sec. The addition of α-ketoglutarate, succinate or glutamate, on the other hand, caused a sharp decrease in oxidized diphosphopyridine nucleotide without a corresponding increase in its reduced form, indicating an increase in the diphosphopyridine nucleotide intermediate. Thus this energy-rich intermediate breaks down in the presence of β-hydroxybutyrate and accumulates in the presence of α-ketoglutarate, succinate or glutamate in the absence of phosphate acceptor.

The interconversions of the various forms of diphosphopyridine nucleotide in rat-liver mitochondria may be explained by the formulation of respiratory-chain phosphorylation currently used in this laboratory[3,8]. Substituting reduced diphosphopyridine nucleotide for AH_2, flavoprotein (*fp*) for *B* in the general formulation we have:

(1) DPNH + *fp* + *I* ⇌ DPN ~ *I* + *fp*H_2

(2) DPN ~ *I* + *X* ⇌ *X* ~ *I* + DPN

(3*a*) *X* ~ *I* + P_i ⇌ *X* ~ P + *I*

(3*b*) *X* ~ P + ADP ⇌ ATP + *X*

(4) *X* ~ *I* + $H_2O \xrightarrow{(DNP)}$ *X* + *I*

(The following abbreviations are used: P_i, inorganic phosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; DNP, 2,4-dinitrophenol; DPN, diphosphopyridine nucleotide.)

It can be seen from reactions (1) and (2) that both free *I* and *X* are needed to convert reduced diphosphopyridine nucleotide and diphosphopyridine nucleotide intermediate, respectively, to oxidized diphosphopyridine nucleotide. The addition of 2,4-dinitrophenol causes a rapid conversion of reduced diphosphopyridine nucleotide and the intermediate form to diphosphopyridine nucleotide because it produces both *I* and *X* in the hydrolytic reaction (4). The addition of adenosine diphosphate also causes a rapid appearance of oxidized diphosphopyridine nucleotide (but slower than that caused by addition of 2,4-dinitrophenol) because it produces free *X* in reaction (3*b*). It is likely that there is sufficient free phosphate in the mitochondria preparation so that reaction (3*a*) can proceed to the right. The ability of phosphate, which in reaction (3*a*) liberates *I* from *X* ~ *I*, to cause the appearance of diphosphopyridine nucleotide may be explained by assuming a slight uncoupling or hydrolysis of *X* ~ *I* to yield free *X*.

The decrease in oxidized diphosphopyridine nucleotide and the increase in diphosphopyridine nucleotide intermediate but not in reduced diphosphopyridine nucleotide that occur on the addition of α-ketoglutarate or glutamate to liver mitochondria are explained by the reduction of diphosphopyridine nucleotide by the mitochondrial dehydrogenase followed by reaction (1). The increased amounts of both oxidized and reduced diphosphopyridine nucleotide that occur on the addition of β-hydroxybutyrate in the presence of magnesium is attributed to a reduction of diphosphopyridine nucleotide by the β-hydroxybutyric dehydrogenase followed by reaction (2). The free *X* needed for reaction (2) most likely results from a slight uncoupling by β-hydroxybutyrate.

The decrease in diphosphopyridine nucleotide with succinate is somewhat surprising since diphosphopyridine nucleotide is not a member of the succinic oxidase system. The increase in diphosphopyridine nucleotide intermediate but not reduced diphosphopyridine nucleotide may be explained by the formation of *X* ~ *P* in the phosphorylation steps associated with succinate oxidation and the reversal of the high-energy-transfer steps associated with diphosphopyridine nucleotide (reactions 3*a* and 2) to form the diphosphopyridine nucleotide intermediate.

The conversion of reduced diphosphopyridine nucleotide to the energy-rich intermediate and the inhibition of diphosphopyridine nucleotide appearance by 0·8 m*M* amytal in the presence of adenosine diphosphate may be explained by the blocking of reaction (2) but not reaction (1) with low concentrations of amytal. Higher concentrations of amytal (1·8 m*M*) also block reaction (1). Thus, it is postulated that the primary site of amytal action is reaction (2).

I wish to express my thanks to Prof. E. C. Slater for his interest and helpful criticism, and to Miss Jet Moerdijk for her skilled technical assistance. This investigation was carried out during the tenure of a Life Insurance Medical Research Followship.

Further details will be published later.

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GENETIC CONTROL OF THE CYTOLOGICALLY DIPLOID BEHAVIOUR OF HEXAPLOID WHEAT

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COMMON wheat, *Triticum vulgare*, is a hexaploid species with 42 chromosomes in which the genomes of diploid wheat and of the diploid species *Aegilops speltoides* and *Aegilops squarrosa* are combined together[1-5]. There is considerable similarity between the chromosomes of these three diploids. Indeed, in hybrids between diploid wheat and *Aeg. squarrosa* and between diploid wheat and *Aeg. speltoides*, complete pairing at meiosis occasionally results in the formation of seven bivalents, although the mean bivalent frequency is somewhat less, being between three and four per cell[5,6]. Moreover, the attraction which results in bivalent formation in the hybrids is of such strength as to cause multivalent formation in tetraploids induced from them. However, each chromosome normally conjugates with its complete homologue at meiosis in *T. vulgare*, and only bivalents are formed. Thus, the pairing attractions between the equivalent, homoeologous, chromosomes of different genomes either no longer exists or is no longer expressed. Polyploid wheat has therefore evolved to behave cytologically as a diploid. Further, the shift has been of such efficiency that there is very little chromosome pairing even in 21-chromosome haploids of *T. vulgare*, in which intergenome pairing is not restricted by the affinity of complete homologues. The cytological distinctiveness of the equivalent chromosomes of different genomes is all the more striking in view of the close genetic relationships which have enabled Sears[7], by nullisomic-tetrasomic compensation, to recognize the seven homoeologous groups into which the complement of wheat can be arranged.

Recent results with various aneuploids have revealed something of the genetic control of the diploid behaviour of *T. vulgare*. The evidence centres on the derivatives of a 41-chromosome monosomic line, designated *HH*, which arose at Cambridge in the variety Holdfast. The formation of twenty bivalents and one univalent (Fig. 6) in *HH* monosomics is like the behaviour of all other monosomics of common wheat[8]. Meiosis is normal in the 42-chromosome segregants in the monosomic line, and in the 42-chromosome hybrids from crosses of *HH* monosomics to euploid plants of Holdfast. Therefore, the chromosomes of *HH* monosomics are structurally unaltered compared with those of Holdfast. One arm of the chromosome monosomic in the *HH* line is rather more than twice the length of the other, but the chromosome has not yet been positively identified relative to the numbering based on the variety Chinese Spring.

Monosomics of *HH* have been used in crosses with 44-chromosome plants which had the full complement of wheat chromosomes plus a single pair of rye chromosomes. From these crosses five 20-chromosome, nulli-haploid, plants have been obtained, which, by the nature of their origin, must have had the haploid complement of Holdfast minus the chromosome which is monosomic in the *HH* line. The nulli-haploids arose in (*a*) the F_1 of the cross *HH* monosomic × rye chromosome III disomic addition, and in (*b*) the F_2 of crosses of *HH* monosomic × rye II, or rye III, disomic additions[9]. All the F_1's which produced nulli-haploids in F_2 had twenty bivalents composed of wheat chromosomes and the *HH* chromosome and a rye chromosome as univalents. In each situation, therefore, the 20-chromosomes in the embryo sacs which functioned parthenogenetically must have been those which had segregated from bivalents—that is, all the wheat chromosomes except *HH*.

The meiotic pairing of the *HH* nulli-haploids has been compared with the pairing in 21-chromosome euhaploids of Holdfast. The meiotic behaviour of the euhaploid plant listed in Table 1 was similar to that of twelve other 21-chromosome plants of Holdfast examined over a period of four years. The number of bivalents in these plants never exceeded four, with a mean frequency of between 1·3 and 1·7 per cell, and trivalents were rare (Fig. 1). There was usually only one chiasma per bivalent.

By contrast, in the *HH* nulli-haploid enumerated in Table 1, which is typical of five such plants examined in two successive years, the mean bivalent frequency exceeded the maximum observed in euhaploids. Trivalents were quite frequent with as many as five in some cells (Fig. 3), although there was never more than one per cell in the euhaploids. Very many more chromosomes per cell, a mean of 11·0 compared with a mean of 2·9, were involved in chiasma-associations and a much higher frequency of closed bivalents and of 'pan-handle' trivalents resulted from more intimate pairing. As many as nineteen of the twenty chromosomes of the nulli-haploid have been observed in various associations simultaneously, although never more than nine were involved in associations in the same cell in euhaploids.

The most reasonable hypothesis to account for these observations was that the deficient chromosome carried a gene, or genes, which restricted

Table 1. Meiotic Pairing in Euhaploid and *HH* Nulli-haploid of *T. vulgare*

Type	Chromosome No.	Cells	Mean pairing			Proportion of bivalents as rings	Conjugated chromosomes per cell	
			Bivalents	Trivalents	Quadrivalents		Mean	Range
Euhaploid	21	100	1·38 ± 0·09	0·07	—	0·00	2·86 ± 0·23	0–9
Nulli-haploid	20	75	4·16 ± 0·12	0·86 ± 0·03	0·02	0·23	10·95 ± 0·32	4–19

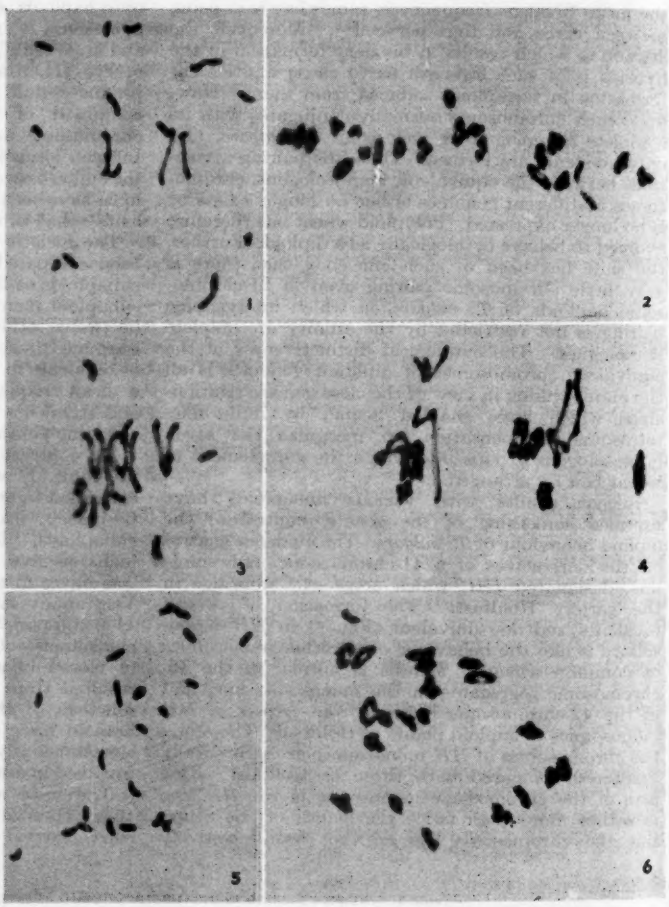
intergenomic, homoeologous, pairing; the alternative being that association between randomly distributed duplicate segments was normally inhibited. On either view, there being no other independent mechanism, in the absence of the pairing restriction normally undetected affinities were expressed. Crucial evidence that the pairing was homoeologous rather than random was afforded by the high frequency of trivalents and the infrequency of quadrivalents.

If homoeologous pairing could take place in *HH* nulli-haploids, it was argued that 40-chromosome nullisomics deficient for the *HH* pair should deviate from the strictly bivalent-forming regime of *T. vulgare*. Consequently, three *HH* nullisomics have been examined. All had large and frequent multivalents, and associated univalents, at meiosis. No method has yet been found of consistently preparing well-spread pollen mother cells of this material, but the complexity of the behaviour is readily apparent (Fig. 4). More than half the cells have at least one multivalent, usually a quadrivalent, and many have several. Associations of three, four, five and six are common, but no higher multivalents have been observed. However, the greater the number and size of multivalents the less is the chance of disentangling the snarl, so there is danger of observational bias. No doubt, also, there are homoeologous, or allosyndetic, bivalents which would be undetected unless markedly heteromorphic. The magnitude of the meiotic disturbance is therefore hard to assess; nevertheless, it is clear that, in the absence of the *HH* chromosome, *T. vulgare* ceases to be a stable bivalent former, a classical example of the autosyndetic pairing allohexaploid, and behaves as an intermediate, autoallopolyploid.

The diploidizing mechanism is effective in the hemizygous state in monosomics and in euhaploids. Furthermore, in a haploid plant which had twenty normal wheat chromosomes plus the iso-chromosome formed from the long arm of the *HH* chromosome (Fig. 5), meiotic pairing was the same as in euhaploids. Moreover, there was no multivalent formation in a 41-chromosome monosomic which had twenty normal pairs of Holdfast chromosomes and in which the *HH* chromosome was represented only by a single telocentric of the long arm. The long arm alone is thus effective in prohibiting homoeologous association; but no evidence is available on the effects of the short arm alone. It may be that both arms have genes with equally effective control over the pairing behaviour, but this seems unlikely. The control of bivalent formation is probably restricted to one arm, and may indeed be effected by quite a localized region.

The implications of these results are both theoretical and practical. First, there has been considerable discussion on the methods by which many polyploids have attained their cytologically diploid character, with its obvious advantages in fertility and genetic stability. Several authors have favoured the theory that this situation has been achieved by the selective accumulation of many small changes of chromosome structure, which lead to the divergence of homoeologues. However, the selection of the localized product of a single mutational step, such as seems likely to have happened in wheat, is very much simpler to envisage, and would clearly be a more rapid and efficient process. To compare the frequencies of the genetical, or cytological, determination of diploid meiotic behaviour, other allopolyploid species should be examined for evidence of a mechanism similar to that in wheat.

Secondly, knowledge of the pairing restricting mechanism may be useful in wheat improvement.



First metaphase of meiosis in Feulgen- and orcein-stained squashes of pollen mother cells of various derivatives of *T. vulgare*

(1) 21-Chromosome euhaploid with 4 bivalents (one widely stretched) and 13 univalents. (2) Euploid with 21 bivalents. (3) 20-Chromosome *HH* nulli-haploid with one 'pan-handle' and 4 chain trivalents, one bivalent and 3 univalents. (4) 40-Chromosome *HH* nullisomic with one ring of six, 2 chains of four and 13 bivalents. (5) 21-Chromosome haploid in which the *HH* chromosome is an isochromosome of the long arm; one stretched bivalent, 18 normal univalents and the isochromosome paired interbrachially with two chiasmata. (6) 41-Chromosome *HH* monosomic with 20 bivalents and one univalent. (×770)

The utilization of related diploid species, for example, in the genus *Aegilops*, in wheat breeding is hampered because the chromosomes of the diploids do not pair with those of wheat, in intergeneric hybrids. To overcome this obstacle, Sears[10] was forced to use X-rays to induce the translocation of a disease-resistance gene of *Aeg. umbellulata* on to a wheat chromosome, and others[9,11,12] have been compelled to explore alien chromosome addition and substitution lines. However, this intergeneric allosyndetic pairing may well take place in the absence of the *HH* chromosome, just as it does between chromosomes of the different genomes; alien genes could then be introduced into wheat chromosomes by normal recombination. Critical hybrids of this constitution can be extracted from crosses of *HH* monosomics by the appropriate diploid.

Finally, nullisomic plants of this constitution may be used either within single varieties or in inter-varietal hybrids in 'intergenome exchange' breeding. Thus a useful gene which showed a dose effect could be obtained duplicated or triplicated, represented on each chromosome of the homoeologous group. The chromosome structure within a variety could be re-patterned by breeding from *HH* nullisomics. Further, if an *HH* nullisomic were also an inter-varietal hybrid, the release of variation, and the range of segregation in later generations, would be very much greater than from a euploid hybrid. Thus, the *HH* nullisomic may afford access to otherwise unavailable genetic variation.

However, the exploitation of *HH* nullisomics depends upon their fertility. There is no natural self-fertility, but a reasonable seed set has been obtained by pollinating *HH* nullisomics with Holdfast euploids, although the reciprocal cross has been unsuccessful. There were several large multivalents at meiosis in the one 41-chromosome derivative, so far examined, of an *HH* nullisomic pollinated by a euploid. The *HH* chromosome regained from the euploid pollen was always a univalent, and homoeologous pairing must have been prohibited by its presence. The multivalents were thus indicative of translocation heterozygosity, the outcome of homoeologous pairing in the nullisomic parent. Homozygotes for new structural conditions can be derived from such plants, although further back-crossing may first be necessary to reduce the extent of structural alteration.

Knowledge of the situation described already begins to make plain some of the problems of wheat cytogenetics and perhaps of polyploidy in general. Further investigation cannot fail to extend this advantage and may well contribute to the cytogenetic manipulation of wheat for practical breeding purposes.

The interest of Dr. G. D. H. Bell in the development of this work is gratefully acknowledged.

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SUCROSE CRYSTAL INCLUSIONS

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TWO years ago I reported[1] the presence of water inside the sucrose crystal as a normal phenomenon, the percentage varying between wide limits. Measurements of vapour pressure, taken before and after crushing, demonstrated this qualitatively, but at that time no adequate quantitative analytical procedure was available for precise measurement. Two new methods have since been developed in our research laboratories[2]. The evidence obtained so far indicates that with refined sucrose crystals of the normal size of granulated sugar, the internal water ranges at least from 0·01 to 0·04 per cent, while with large crystals approaching an inch in length, the range of internal water may be ten times greater (0·1 to 0·4 per cent).

Since this included water is due to the 'growing in' of mother syrup by the layers during the growth of the crystal[1], the composition of this internal syrup will be essentially that of the mother syrup.

Given the composition of the mother syrup, together with the amount calculated from the included water, it is possible to estimate the amounts of the various non-sucrose constituents held in syrup inclusions.

Results obtained so far suggest that water-soluble organic colouring matter is held entirely in these syrup inclusions, but that certain ionized salts are frequently found in far higher proportion than that calculated on the basis of included syrup. Calcium and potassium salts seem to be thus held, and in particular, the potassium salt of imidodisulphonic acid[3], and calcium magnesium aconitate in beet and cane sugars respectively. It is not known whether these are deposited as crystals which are then built-in by the layer growth, or whether they are held by some ionic or molecular similarity of structure with some part of the sucrose molecule.

Non-crystalline insoluble matter may also be 'grown in' by the layer growth, as, for example, starch, which is found in the sap of certain strains of sugar cane. This appears to be attracted towards the growing crystal face, since after several successive crystallizations, the starch is found in the crystals in a higher ratio to soluble salts than in the original juice. Certain colloids appear to act similarly.

Further study of sucrose crystals under the microscope has also revealed gaseous inclusions. By watching chosen specimens in the act of dissolving under the microscope, when an inclusion of syrup is breached, the heavy syrup may be seen streaming downwards, whereas when a gaseous inclusion is breached a bubble may be seen to strain like a

balloon, then to break away and rise rapidly to the surface. The probable origin is that air dissolved in the original crystallizing syrup became supersaturated and formed as bubbles on the growing face. These were then overgrown by the layers. One obvious effect of these inclusions is to explain the widely varying figures given in the literature for the specific gravity and melting point of sucrose—1·58–1·60 gm./cm.^{3} and 160–186° C., respectively. By selecting two crystals under the microscope with maximum and minimum visible inclusion and determining their specific gravities, we have obtained figures of 1·582 and 1·594 gm./cm.^{3} respectively.

These fluid inclusions take several distinctly different physical forms (see Figs. 1–5).

(1) A fjord-like form, the position of which in the crystal appears to be completely random, though frequently several are present roughly parallel to one another.

(2) A rather symmetric form, of which the most perfect and complete is usually referred to as the 'hour-glass' shape, since when viewed toward the 100 face, the familiar hour-glass shape is apparent. Sometimes only a section of this may be present.

(3) Orderly patterns indicating some symmetry—sometimes, but certainly not always, the result of *Einwurf* boiling (using crystals from one boiling as seed for a second).

(4) Veil-like systems, in appearance suggesting a wisp of gossamer flicked through the crystal, with no respect for the axis.

(5) Local clouds or drifts of approximately spherical droplets in diameter from 100μ down at least to 1μ.

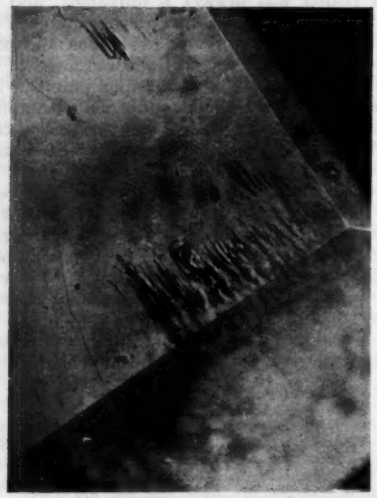

Fig. 1. Fjord-type inclusions (note also layer growth)

Fig. 2. Hour-glass inclusion

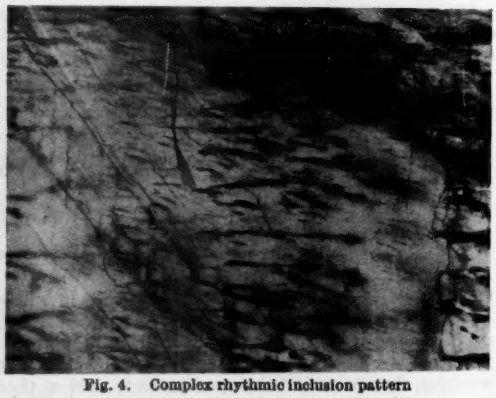

Fig. 4. Complex rhythmic inclusion pattern

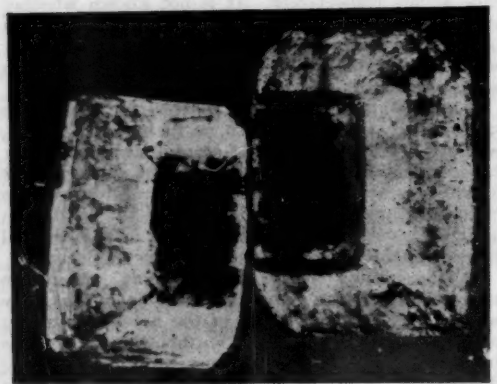

Fig. 3. *Einwurf* inclusion, showing evidence of lower growth-rate in one direction

Fig. 5. Veil, showing hour-glass inclusions

The evidence suggests that the first three are the result of layer growth 'building-in' liquor or air locally, and a fortunate micro-film shows the fourth to be the result of a sudden crack, due to growth or temperature strain. Whereas the initial veil consists of a continuous film layer, this appears to be unstable, and I am fairly confident that, if conditions of temperature and time permit, the film slowly 'congeals' to a more stable condition. The first stage reminds one of a network of isthmuses; the later stage is the network of detached droplets. This I have classed as the distinct type 5, since it is possible that some of these droplets may have been 'grown in', as with type 1.

It is interesting that layer growth is able to proceed around such dissimilar foreign intrusions as thread, wire, sheets of metal, local spots of liquor at lower supersaturation and even bubbles of air, and that without apparent disturbance to the crystal symmetry. Dr. A. F. Seager originally directed my attention to the fact that the major inclusions are normally to be found in that portion of the crystal which has grown most rapidly, in conformity with the usual belief that rapid growth increases the tendency to form inclusions. I have now found many instances of this phenomenon.

Another lucky micro-film suggests that one part of one face of a crystal may exhibit a local 'spurt' in the rate of growth. This may play a part in the formation of the larger inclusions. Artificial inclusions have been made successfully by boring a hole through a large crystal, then immersing it in a supersaturated solution, when the two exits may be sealed, thus closing the liquor-filled tunnel.

The evidence, both with mosaic and with the many types of inclusion, indicates that the interior of sucrose crystals normally exhibits great local variation in structure.

So much crystallographic research is in the nature of a post-mortem upon geological crystals formed in the distant past. It would appear that the study of such a crystal as sucrose in active growth should provide the key to several features common to many crystalline materials.

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USE OF TWO PROPERTIES OF THE VIRUS OF FOOT-AND-MOUTH DISEASE IN STUDIES OF ITS MULTIPLICATION

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IN a previous study of the multiplication of the virus of foot-and-mouth disease in cultures of tongue epithelial cells[1], it was found that infective virus remained bound to the cells during the latent period, that is, up to 2–2½ hr. after infection even after treatment of the cells with specific antiserum. The number of infective units of virus (*ID*50/ml.) was approximately equal to the number of cells producing virus; it was therefore not possible to decide whether this infectivity was due to virus other than that producing infection, adsorbed in such a way that it resisted the action of antiserum, or to virus which after entering and infecting the cell remained infective. Since in studies of phage and other viruses[2–6] the infecting virus has been shown to become non-infective after penetration, it was important to decide between these possibilities.

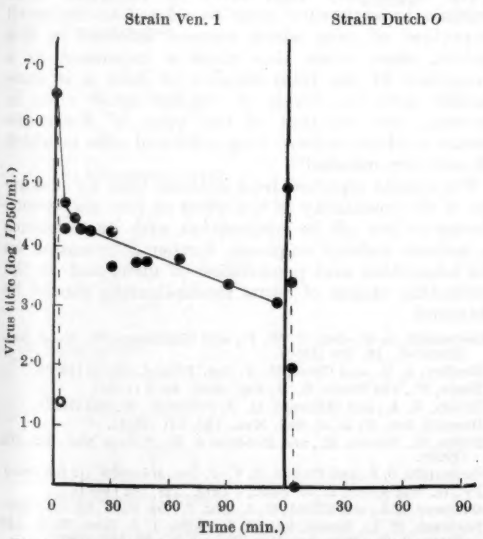

Fig. 1. The *p*H-inactivation of the Ven. 1 and Dutch *O* strains of the virus of foot-and-mouth disease; ●—●, *p*H 6·0; ○—○, *p*H 5·7

Two properties of the virus of foot-and-mouth disease have been utilized for this purpose. In a paper by Cartwright and Thorne[7], the resistance of the virus to sodium dodecyl sulphate and other detergents is described, with its application to the lysis of tongue epithelium and pig kidney cells and extraction of virus throughout the growth-cycle. Sodium dodecyl sulphate has advantages in this type of work over other methods of disrupting cells in the rapidity and completeness of its action. With this reagent and again using antiserum to inactivate extracellular virus, infectivity of the same order was still found during the latent period with both cell types.

Secondly, the well-known instability of this virus at *p*H values below neutrality[8–10] suggested lowering the *p*H of the culture as an alternative means to the use of antiserum or washing. The possible advantages of *p*H change are the rapidity and completeness of the inactivation, and the ease of penetration of the hydrogen ion into the cell surface. The results of using *p*H inactivation in conjunction with the sodium dodecyl sulphate method of disruption to determine virus remaining associated with the cells are reported here.

The characteristics of the inactivation of the two strains of virus used, Ven. 1 and Dutch *O* (1957), at

relevant *p*H values are shown in Fig. 1. At *p*H 6·0 the Ven. 1 strain is inactivated at a slower rate than Dutch *O* and in addition a small fraction of the virus population (~ 1 per cent) is inactivated at a much slower rate than the main fraction. A similar fraction was found in the strain studied by Bachrach *et al.*[10]. At *p*H 5·7, however, the rate of inactivation is such that 99·9 per cent of both Ven. 1 and Dutch *O* virus is inactivated in less than 1 min.

In the experiments with cell suspensions the following procedure was used. Suspensions of tongue epithelial and pig kidney cells were exposed to seed virus (1–100 *ID*50/cell) for 30 min. at 37° C., when the suspension was adjusted from the normal *p*H (7·6) to *p*H values in the range 5·0–6·0 either by sedimenting the cells and resuspending in 0·02 *M* acetate-buffered medium (Tyrode's + 0·5 per cent lactalbumin hydrolysate) or rapidly by the addition of 0·1–1·0 *M* acetic acid. After 5–70 min. the *p*H was restored to 7·6 either by sedimenting the cells and resuspending in normal medium or by the addition of 0·01 *N* sodium hydroxide or *p*H 7·6, 0·04 *M* phosphate buffer. A portion was then titrated by inoculation of unweaned mice[11] to determine the number of infected cells, and a portion, treated with an equal volume of 1·0 per cent sodium dodecyl sulphate in phosphate buffer to destroy the cells, was titrated to determine residual infective virus. The remainder was incubated at 37° C. and titrated to determine the yield of virus after multiplication.

From the representative results in Table 1, the virus associated with the cells after infection with the Ven. 1 strain of virus is now seen to be of the order of 1 infective unit/100 infected cells, while the virus produced remains normal, within the limits of error of titration (± 0·3 log unit), for *p*H treatments at 5·5 and above up to 15 min. Below *p*H 5·5 most of the cells become non-viable in the medium used. Longer times at low *p*H appear to slow down the multiplication of virus, although the eventual yield is normal; this is now being investigated.

Table 1. Latent Period Infectivity. Strain Ven. 1 in Tongue Epithelial Cells

*p*H	Log No. cells/ml.	Infected cell titre Log No./ml.	Per cent ratio cell-associated virus / Infected cell titre	Yield *ID*50/ml.
6·0	6·3	5·1	0·5	7·6
5·9	6·6	5·0	1·0	—
5·7	6·7	4·9	1·0	7·6
5·5	6·8	5·3	0·1	7·8

When the virus is kept in contact with the cells for times less than 30 min. before rapid adjustment to low *p*H (5·7), the cell-associated virus at short adsorption times is reduced still further (Fig. 2), to about 1 infective unit/10^4 infected cells, although the number of infected cells is the same within the experimental error after 1 min. as after 30 min. adsorption. There is a steady increase in the cell-associated virus with time of adsorption until the normal level after 30 min. adsorption is reached. No increase in the cell-associated virus with time was observed with the Dutch *O* strain of virus. It is possible that the greater cell-associated infectivity with the Ven. 1 strain is related to the heterogeneity of this strain towards *p*H inactivation.

These experiments show that the virus of foot-and-mouth disease becomes non-infective on entering the cell. A rough estimate of the rate at which virus

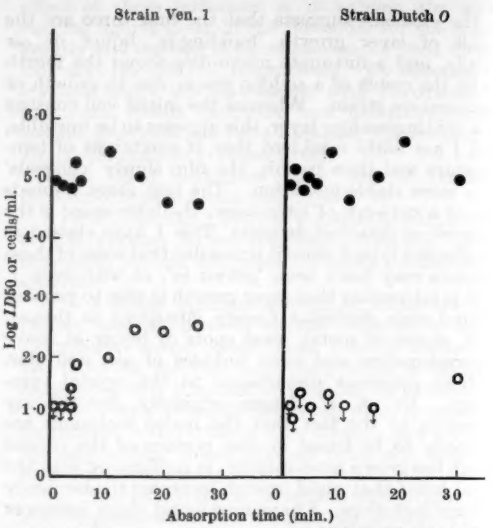

Fig. 2. The number of infected tongue epithelial cells and titre of cell-associated virus resulting from adsorption of the Ven. 1 and Dutch *O* strains of the virus of foot-and-mouth disease at 37° C. (seed virus $10^{7\cdot6}$ *ID*50/ml.; cells $10^{6\cdot6}$ ml.; ↓ represents titre ≤ that indicated). ●, Infected cells; ○, cell-associated virus

enters the cell and becomes non-infective can also be made. The number of infected cells after an adsorption time of 1 min., followed by lowering the *p*H, was shown to be of the same order as after a 30 min. adsorption period. Determination of the Dutch *O* strain virus surviving after increasing times at *p*H 5·7 showed that the extracellular and adsorbed virus was reduced from a total of about $10^{7\cdot7}$ *ID*50/ml. to $10^{3\cdot5}$ *ID*50/ml. (1 *ID*50/100 infected cells) in less than 30 sec. In addition, the action of sodium dodecyl sulphate is so rapid that no appreciable penetration could occur after *p*H treatment. It can therefore be concluded that adsorption and penetration must occur in less than 1 min. at 37° C.

The apparently high level of adsorbed virus resistant to antiserum[1] may be related to the small proportion of cells which become infected in this system, since when this virus is expressed as a proportion of the total number of cells it is comparable with the levels of cell-associated virus in systems, such as that of the virus of Newcastle disease in chick embryo lung epithelial cells in which all cells are infected[12].

The results reported here indicate that by making use of the instability of the virus of foot-and-mouth disease at low *p*H in conjunction with its resistance to sodium dodecyl sulphate, further information on the adsorption and penetration of virus and on the succeeding stages of virus multiplication should be obtained.

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LETTERS TO THE EDITORS

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Carcinogenic Activity and O-Alkylation

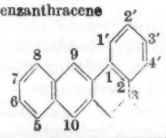
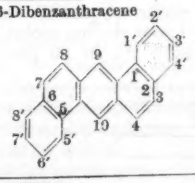
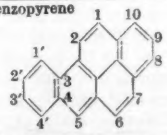
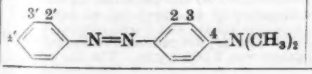
It is well known that many natural products, flower pigments, essential oils, alkaloids, etc., contain methoxy-groups, and that some of these compounds show remarkable biological activities, for example, colchicine, mescaline, etc. Despite the enormous amount of work done on the correlation of biological activity with the chemical structure, surprisingly little attention has been devoted to the biological effects connected with O-methylation of phenolic groups (compare, for example, Sexton[1]). Yet examples of its influence have been known for a long time (for example, morphine and codeine). The situation is likely to change in view of the recent discovery that O-methylation can occur in the animal body[2]; the reverse process, O-demethylation was observed in mammals about twenty years ago[3]. In view of the finding that the methyl group of methionine is also involved in O-methylation[4] the knowledge acquired from the study of N-methylation will no doubt help in the understanding of the interrelation of these two types of reactions.

With reference to carcinogenic activity, I was first impressed by the decisive part which O-methylation can play when the methylation product of the almost inactive phenolic metabolite of 3 : 4-benzopyrene produced tumours in mice[5]. Pure 8-methoxy-3 : 4-benzopyrene was then synthesized[6] and proved to be one of the most effective carcinogens for the skin and for the subcutaneous tissues of both mice and rats, surpassing in this respect the parent hydrocarbon[7]. Carcinogenic activity, though of a lesser degree, is exhibited by several other isomeric methoxy-3 : 4-benzopyrenes and also by methoxy-derivatives of other polycyclic hydrocarbons[8] (Table 1). The methoxy-groups, as well as the methyl-groups, often increase the efficacy of the hydrocarbon, but the effect depends to a striking degree on the number of the methoxy-groups and on the positions of substitution. The paucity of available data do not yet permit generalizations to be confidently made. Thus, methyl ethers at metabolically oxidized positions are not necessarily the most active: 4'-methoxy-1 : 2-benzanthracene was inactive, while the 3-, 5- and 10-isomers produced tumours.

Restoration or increase of carcinogenic activity by O-methylation is not confined to polycyclic aromatic hydrocarbons, but appears to be more generally encountered[9] (Table 1). Some examples have been reported recently among compounds representing 'remote' carcinogens, which usually induce tumours not at the site of application but in internal organs, such as liver, bladder, etc. It is not unlikely that the actual carcinogens are formed when these compounds are metabolized in the body. Indeed, in the case of some aromatic amines, the metabolic oxidation products, *o*-hydroxamines, proved to be 'local' carcinogens, when implanted in the mouse bladder. Only very few of their methyl ethers have so far been tested, and these appear to be more active than the hydroxy-compounds. The fact that 1-methoxy-2-aminonaphthalene, 3-methoxy-4-aminodiphenyl and 3-methoxy-4-aminostilbene have been reported carcinogenic[9] is of significance. It would be of interest to know whether all these compounds show 'local' carcinogenic activity when applied to tissues other than the bladder.

It is of particular interest that recently some *o*-hydroxamines which are normal intermediary body metabolites, such as 3-hydroxykynurenine, 3-hydroxy-anthranilic acid, and 2-amino-3-hydroxy-acetophenone have been reported to produce tumours

Table 1. Comparison of Carcinogenic Activity of some Hydroxy- and Methoxy-Derivatives of Carcinogens

Compound	Carcinogenic activity	Hydroxy-derivatives Position of substitution	Hydroxy-derivatives Carcinogenic activity	Methoxy-derivatives Position of substitution	Methoxy-derivatives Carcinogenic activity
1 : 2-Benzanthracene 	±	4' 3 8 10	− ± − −	4' 3 5 10 3 : 10-di 9 : 10-di	− ++ ++ +++ − −
1 : 2-5 : 6-Dibenzanthracene 	+++	9 − 3' : 7'-di 4' : 8'-di 2' : 6'-di	− − − −	9 3 9 : 10-di	++ − −
3 : 4-Benzopyrene 	+++	4' 2 5 6 8	− − − + ±	4' 5 6 (or 7) 8 5 : 8-di 5 : 10-di	− ++ ++ ++++ − −
4-Dimethylaminoazobenzene 	+++	2' 3' 4' 2 3	− − − − −	2' 3' 4'	++ +++ ++

when tested by the bladder implantation method. The 8-methyl ether of xanthurenic acid was again more effective than the latter, 4 : 8-dihydroxyquinoline-2-carboxylic acid[9].

More data are urgently needed on the biological activities of methyl ethers of phenolic compounds normally present in tissues, and on the conditions which may affect the equilibrium between metabolic O-methylation and O-demethylation—evidently of paramount importance for carcinogenic activity.

The evidence already available is sufficient to warrant further exploration for carcinogenic activity also of other alkyl ethers of such phenolic compounds. In this connexion the finding of carcinogenic activity among some alkylating agents is of interest. These alkylating agents have been studied in connexion with the cross-linkage hypothesis of carcinogenesis[10]. Their role as agents which could alkylate phenolic compounds deserves to be considered.

I wish to thank Dr. J. W. Cook and Dr. J. M. Barnes for very valuable discussions.

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Carnosine Phosphate as Phosphate Donor in Muscular Contraction

Under the above title Goodall[1] showed that a mixture of carnosine di- and mono-phosphates has a relaxing action practically identical with that of creatine phosphate on the glycerol-extracted muscle fibre. He raised the question of the presence and role of this substance in muscle, and pointed out that its non-detection so far may be because it is so much more acid-labile than creatine phosphate[1,2].

In order to provide the energy for the 125 gm. cm. of external work done per gram of muscle in a typical twitch a 'high-energy' phosphate compound would have to be present at a concentration of at least 0·25 μmole per gm. muscle. (This calculation takes a low value for the external work done, neglects the heat produced in the muscle, assumes 100 per cent efficiency in energy transduction and a high value (12,000 cal.) for the energy delivered per mole of compound broken down.) A more probable figure would be 0·5 or more μmole per gm. muscle[3,4]. Since preliminary experiments showed that large amounts of carnosine phosphates were not present in muscle the following procedure was employed. The very slow, thin, paired, striated, retractor penis muscles of turtles were used. This muscle can be frozen in the relaxed state without change in length, and measurements of the longitudinal electrical conductivity with thermally insulated electrodes showed that the centre of the muscle was frozen in less than one-fifth of the time needed for a normal contraction at 0° C. This means that there was no time for a possible isometric contraction of the internal parts of the muscle to occur during freezing, which might have vitiated the results. The muscles were immersed at 25° C. for a total of 4 hr. in a physiological salt solution containing radioactive inorganic orthophosphate. After 2 hr. they were stimulated electrically several times and allowed to recover. This procedure causes a chemical turnover of all phosphate compounds involved in muscle contraction and ensures a close approach to equality in the specific radioactivities of the labile phosphate compounds in this tissue[5]. Thus if carnosine phosphates were present in even as low a concentration as 0·25 μmole per gm. muscle the total radioactive counts per minute in this compound would have to be at least 0·25/13, that is to say, 0·019 times the total radioactivity in the creatine phosphate which is present at a concentration of 13 μmoles per gm. muscle.

At the end of the incubation period the muscles were attached to isotonic levers, cooled to 0° C., frozen in the relaxed state in melting dichlorodifluoromethane (− 155° C.), and powdered at − 198° C. Carrier quantities of a mixture of non-radioactive synthetic carnosine di- and mono-phosphates[3] were added to preserve any such radioactive material in the tissue from possible losses during isolation. The powder was extracted at 0° C. in 20 per cent v/v

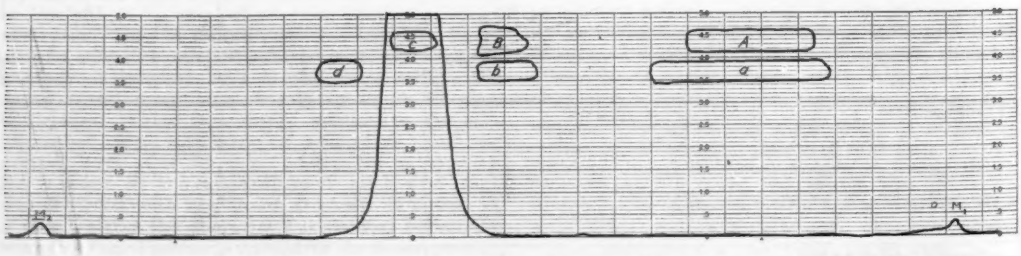

Fig. 1. Distribution of radioactivity in muscle extract treated with barium. *A*, *B* and *C* correspond to the positions on the chromatogram of carnosine diphosphate, carnosine monophosphate and creatine phosphate respectively as shown by an acid-molybdate spray for phosphorus. *a*, *b* and *d* correspond to carnosine diphosphate, carnosine monophosphate and tissue carnosine as shown by a spray of *p*-nitrobenzenediazonium fluoborate after treatment with acid *in situ*. *M* and M_1 are radioactive marker spots; *O* is the origin. Full-scale deflexion of the scanner at the sensitivity used for this graph was 3,000 c.p.m. The R_F of the creatine phosphate is 0·56

ethanol/water and the radioactive inorganic phosphate and nucleotides removed from the extract by repeated isotope dilution and precipitation with barium at *p*H 8·2. The solution was then chromatographed on paper with a mixture of 65 volumes of 95 per cent v/v ethanol/water and 35 volumes of 0·1 *M* potassium carbonate. Examination of the paper with an automatic radioactivity scanner and with sprays for phosphate and imidazole compounds gave the results shown in Fig. 1. Although the added carnosine phosphates had survived the isolation procedure there was a complete absence of associated radioactivity. The creatine phosphate contained 18,000 c.p.m., so the carnosine phosphates should have had at least 18,000 × 0·019 = 340 c.p.m. However, the radioactivity attributable to the carnosine phosphates was less than 30 c.p.m. A similar absence of carnosine phosphates was found in these muscles contracted at 0° C. under conditions where it had previously been found that net changes in adenosine tri-, di- or mono-phosphates and in creatine and creatine phosphate were either unobservable during a contraction or too small to account for the work done[3,4,6].

These results appear to exclude the possibility that the carnosine phosphates in the Severin, Georgievskaya and Ivanov preparation[2] are phosphate donors in muscle contraction and show that, if present at all, their concentration in muscle must be less than 0·02 μmole per gm.

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Deoxycytidine in Urine as an Indicator of Changes after Irradiation

Ionizing radiation causes obvious changes in the metabolism of deoxyribonucleic acids[1]. An experiment was therefore carried out to determine whether exposure of rats to ionizing radiation causes excretion of catabolites or intermediary products of deoxyribonucleic acids in urine.

Adult white male rats (Wistar strain) weighing 200–320 gm., kept under standard conditions, were used. The animals were irradiated with 76 r./min. at a distance of 40 cm. at 200 kV., 15 m.amp. with a filter consisting of 0·5 mm. of copper and 1 mm. of aluminium. Urine was collected from unrestrained rats kept in glass metabolic cages, making possible the separate collection of urine and stools, and from animals partly restrained in metabolic cages.

Dische's reaction with cysteine and 75 per cent sulphuric acid in Stumpf's modification[2] was applied

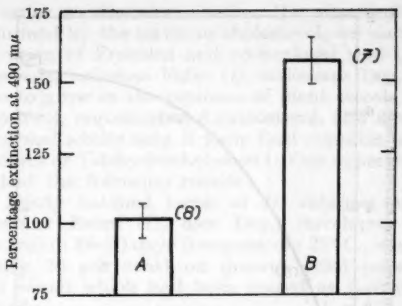

Fig. 1. The effect of irradiation on the excretion of Dische-positive substances in urine. Extinction at the wave-length of maximum absorption (490 mμ) in the urine of *A*, control rats; *B*, irradiated animals. Urine was collected during the first 24 hr. after irradiation with a dose of 300–600 r. and adjusted to a constant volume

to an equivalent amount of filtered urine. The spectral character of the colour formed was determined, together with the extinction at the wave-length of maximum absorption. For qualitative evaluation substances isolated from the urine with active charcoal were analysed by paper chromatography and detected using absorption in ultra-violet light and the reaction for deoxyribose and its compounds[3]. R_F values for the substances detected and standards were compared and absorption spectra determined for identification purposes. For quantitative data an equivalent amount of urine analysed chromatographically on Whatman paper No. 3 and substances containing deoxyribose were determined in an aqueous eluate using Dische's reaction[2].

In the first experiments a total dose of 300–600 r. was applied. It is evident from Fig. 1 that irradiation causes an increased excretion of substances containing deoxyribose. The colour that arises has a characteristic maximum of absorption at 490 mμ. This increased excretion of substances containing deoxyribose is absent in the urine of animals stressed in other ways (partial restraint, multiple fractures of both hind limbs, injection of formaldehyde into the plantar region of both hind limbs, traumatization in the Noble–Collip drum and burns of the third degree in the lumbar region). This increased excretion of substances containing deoxyribose after irradiation was observed both in animals in metabolic cages fed with normal diet and in those animals that after irradiation had no access to food for the whole period of collection of urine. The excretion of these substances was also found after irradiation of female animals. Further work is required to determine whether there are quantitative differences in the amount excreted between the sexes.

An attempt was made to identify the substances containing deoxyribose that are excreted in increased amount in the urine after irradiation. Using the method described to isolate the substance it was possible to identify it as deoxycytidine on the bases of R_F values, positive reaction for deoxyribose, release of cytosine by acid hydrolysis and spectral characteristics.

It was further necessary to determine the amount of deoxycytidine thus excreted and its relation to the dose of irradiation applied. In addition, the excretion of the deoxyriboside of the other pyrimidine base of deoxyribonucleic acid—thymidine—was also studied.

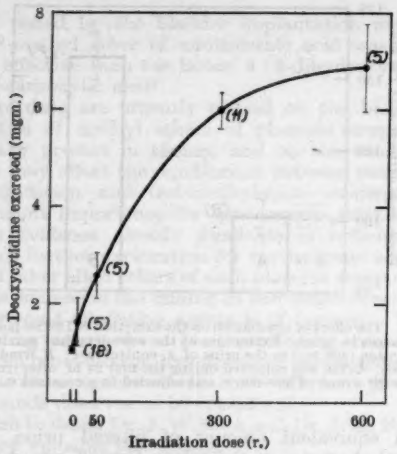

Fig. 2. Relation between the amount of deoxycytidine excreted and the irradiation dose. Urine excreted during 24 hr. was adjusted to a constant volume and an aliquot part analysed by paper chromatography. The part of the chromatogram containing deoxycytidine was eluted and deoxycytidine determined in the eluate colorimetrically (ref. 2). Averages and standard errors are shown

Results are summarized in Fig. 2. It could be shown that within a certain dose-range the intensity of excretion of deoxycytidine depends on the dose of irradiation used during the first 24 hr. after irradiation. On the other hand, it was found that irradiation within a certain dose-range has no analogous effect on the amount of thymidine excreted in the urine during the first 24 hr. after irradiation. Values for the latter substance were on the pre-irradiation level.

Excretion of deoxycytidine after irradiation offers new possibilities in the study of the effect of ionizing radiations on metabolism. From that point of view it is thought to be of importance to attack the problem of the causes for release of deoxycytidine after irradiation. Preliminary experiments showed that this excretion is not due to a renal factor, for in nephrectomized animals irradiation results in accumulation of substances containing deoxyribose in the body. This indicates that irradiation causes either increased release of deoxycytidine from deoxyribonucleic acids or that this substance accumulates as a result of a stop in the synthesis of deoxyribonucleic acid.

It is therefore of interest that a corresponding amount of the other pyrimidine deoxynucleoside, thymidine or its compounds, could not be demonstrated in the urine after irradiation. Consequently, in future work the role of thymidine and its derivatives will be studied together with their metabolic fate in relation to the effects of ionizing irradiation.

Excretion of deoxycytidine in the urine after irradiation also offers a new possibility for determining the degree of damage due to ionizing radiation both quantitatively and qualitatively, since within a certain dose-range the intensity of the reaction depends on the dose. Damage can be detected earlier and much more simply than with the diagnostic methods in use at present. It is of importance that excretion of deoxycytidine could also be demonstrated after low irradiation doses.

It may be expected that the excretion of deoxycytidine can thus become a new sensitive indicator of post-irradiation changes. It may further be useful in evaluation of protection against ionizing radiation.

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Anæsthesia and the Action of DDT on *Anopheles* Larvæ

Between 95 and 100 per cent of newly moulted fourth-stage larvæ of *Anopheles quadrimaculatus* Say (LTD strain, Diptera, Culicidae) die within 24 hr. after a 10-min. exposure to massive doses of suspended DDT (1 : 10,000, 1 per cent acetone)[1]. The purpose of this communication is to direct attention to a very remarkable decrease in mortality of newly moulted larvæ to DDT when they have been anæsthetized with ether or carbon dioxide just prior to testing.

In the first series of experiments, 1-hr. old fourth-stage larvæ of *A. quadrimaculatus* were divided into two groups. Those in the first group were briefly rinsed in distilled water and placed individually in glass stoppered vials containing 5 ml. of 3·75 per cent ether in distilled water where they were left for 10 min. They were removed, washed in distilled water and transferred to freshly prepared acetone suspensions of DDT (10^{-4}) by means of fine cardboard tips. Following exposure to DDT, the larvæ were washed in two 1-l. changes of distilled water and finally placed in small numbered observation beakers containing food and water. The still anæsthetized larvæ were carefully oriented at the air–water interface. The second group was not anæsthetized prior to exposure to DDT. In a subsequent series of experiments larvæ were individually exposed to carbon dioxide gas in a small Erlenmeyer flask for 2 min. prior to treatment with DDT for 5 and 10 min., while controls received no treatment with carbon dioxide.

It is apparent from the results in Table 1 that anæsthesia significantly alters the mortality of larvæ to massive doses of DDT. The differences between ether and carbon dioxide are probably related to the fact that ether gives a deeper and much more prolonged anæsthesia.

The protective effect of anæsthesia is not due to failure of anæsthetized larvæ to eat the DDT drop-

Table 1. Effects of Ether and Carbon Dioxide Anæsthesia on the Mortality of 1-hr. old, Fourth Stage Larvæ of *Anopheles quadrimaculatus* Say to DDT (10^{-4}). Four to Eight Larvæ per Test (Average Five)

Treatment	No. of tests	Percentage dead at 24 hr.
Half-saturated ether for 10 min. before DDT for 10 min.	12	17·9
Controls in DDT 10^{-4} for 10 min.	12	100·0
Carbon dioxide for 2 min. before DDT for 5 min.	5	20·0
Controls in DDT for 5 min.	4	56·3
Carbon dioxide for 2 min. before DDT for 10 min.	5	74·0
Controls in DDT for 10 min.	5	96·0

lets, for DDT affects unanæsthetized larvæ far too rapidly for this to be the case. Nor can it be due to the failure of larvæ to encounter a sufficient number of DDT particles since anæsthetized larvæ which are drawn about within the suspension by cardboard tips are entirely comparable to those left completely quiescent.

The results suggest the intriguing possibility that initially DDT is either actively attached to the cuticle or is actively transported across it. Whatever the final explanation, it is clear that ether anæsthesia, in particular, interferes in some important way with the obtainment of a lethal dose of DDT.

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Fate of Beta-Sitosterol in Housefly Larvæ

Twenty different species of insect larvæ, distributed among four orders (Orthoptera, Coleoptera, Lepidoptera and Diptera) have been found so far to require sterols as growth factors. All of them, excepting the Dermestidae (*Dermestes vulpinus* Fabr.[1] and *Attagenus piceus* Oliv.[2]), can utilize both zoosterols and phytosterols; certain species, however, grow faster with dietary cholesterol than with a phytosterol[3]. Furthermore, the activity of the intestinal sterol esterase of *Periplaneta americana* L. was found to be limited to the esters of cholesterol, Δ^7-cholestenol and 7-dehydrocholesterol as the substrate[4]. These observations, together with Bergmann's[5] report on the cholesterol content of the phytophagous silkworm *Bombyx mori* L., led us to suspect that the dietary phytosterols are converted to a zoosterol before utilization. Hence, sitosterol conversion was investigated in the larva of the housefly.

Larvæ of *Musca vicina* Macq. were grown to pupation in an axenic culture with *Escherichia coli*, on a mixture of 40 per cent wheat bran and water. The sterols of the culture medium were identified by the isolation of their acetyl derivatives, which, after recrystallization first from 50 per cent ethanol and then ether, melted at 127·5–128·5° C., the melting point of β-sitosteryl acetate. The ultra-violet spectrum and the slow response to the Liebermann–Burchardt reagent proved the absence of conjugated double bonds. Also, the infra-red spectrum was identical with that of β-sitosteryl acetate.

In order to discover whether the sitosterol was transformed by the larvæ to cholesterol, we used the observation of Fraenkel and co-workers[1] that larvæ of *Dermestes vulpinus* Fabr. (*D. maculatus* Deg.) are unable to grow in the presence of plant sterols such as sitosterol, ergosterol and zymosterol, and develop into normal adults only if their food contains either cholesterol or 7-dehydrocholesterol. Our experiments have had the following results:

(1) Newly hatched larvæ of *D. vulpinus* or *D. cadaverinus* Fabr. (*D. ater* Deg.) developed into adults within 26–30 days (temperature 28° C., relative humidity 70 per cent) on ground dried pupæ of *Musca vicina* which had been reared as mentioned previously.

(2) The dermestid larvæ failed to grow and died in their first instar on a diet composed of 78 per cent ground housefly pupæ which had been extracted exhaustively with acetone and chloroform, of 10 per cent dried yeast extract, 11·5 per cent water and 0·5 per cent Nipagin *M*.

(3) Addition of 0·3 per cent cholesterol to this inactive mixture restored the nutritional value it had before the extraction, while neither the addition of the sterol isolated from the diet of housefly larvæ nor authentic β-sitosterol promoted the growth of the larvæ.

(4) The sterol of the housefly pupæ satisfied the sterol requirement of *D. vulpinus* and *D. cadaverinus*; the larvæ developed into normal hide beetles within 30–33 days on the extracted diet, supplemented with 0·3 per cent sterol of fly pupæ.

These results indicate that the larvæ of *M. vicina* convert, at least to some extent, dietary sitosterol into a sterol which is nutritionally equivalent to cholesterol or 7-dehydrocholesterol in *Dermestes* larvæ.

For the sake of comparison, some experiments have been carried out with powders of dried phytophagous insects, namely, pupæ of *Colias hyale* Godt. collected from various kinds of Leguminosae, and larvæ of *Orgyia antiqua* L. picked from their food plant *Polygonium equisetiforme*. Larvæ of *D. vulpinus* were able to complete their life-cycle on powders of both insects within 25–28 days, thus proving that these insects convert the phytosterols of their diet into zoosterols, utilizable by *Dermestes* larvæ.

Recently, Beck and Kapadia[6] have reported that larvæ of *Tribolium confusum* Duv. are capable of converting sitosterol and ergosterol into sterols resembling, or identical with, cholesterol and 7-dehydrocholesterol. While in this case the ingested sterols were metabolized almost completely, apparently only a small percentage of the sitosterol need be converted into cholesterol by the housefly larvæ. Our experiments indicate that the pupæ still contain considerable amounts of the original β-sitosterol.

It follows that the larvæ of *Musca* and *Orgyia* and the pupæ of *Colias* are capable of eliminating the 'supernumerary' carbon atoms of the phytosterol side-chain and convert the phytosterols to the skeleton of cholesterol. The larvæ of *Dermestes* are incapable of carrying out this reaction. This biological degradation is the more interesting as it has recently been shown[7] that the biosynthesis of ergosterol proceeds via cholesterol, into which the additional methyl group is introduced by means of methionine (the same is true for the methylene-branching in the side-chain of eburicoic acid[8]).

Thanks are due to Pest Infestation Laboratories, Slough, Bucks (England), for a supply of pupæ of

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Cytotoxicity of Heterologous Sera to Normal and Leukæmic Human Lymphocytes

Theoretically, cancer therapeutic agents should be able to differentiate between malignant and normal human cells and should be more toxic to the malignant cells. However, human leukæmic lymphocytes and cancer cells did not show, in *in vitro* studies[1,2], any greater sensitivity than normal cells to X-rays and chemotherapeutic agents. These disappointing results led to tests on the capacity of heterologous sera to discriminate between cells.

The slide chamber method[3] was used to determine the survival of normal and leukæmic lymphocytes in a medium composed of equal parts of test sera and of *TC* 199. The term 'leukæmic lymphocytes' is used to designate the lymphocytes from the blood of patients with chronic lymphogenous leukæmia. 'Normal lymphocytes' were derived from the blood or lymph nodes of non-leukæmic patients, most of whom had only minor disabilities. The serum of each rabbit was tested separately within ten days after bleeding. The number of viable lymphocytes were counted 2 hr. after incubation at 37° C. and once a day thereafter. An early positive toxic reaction was defined as one in which 90 per cent of the lymphocytes were killed in 24 hr.

Table 1. Toxicity of Rabbit Sera and of Inactivated Guinea Pig Sera to Normal and Leukæmic Lymphocytes

Sera	Source of: Lymphocytes	No. of tests	Percentage of tests: 10 per cent survival time (days): 0–1	1–3	3–5	5–14
22 Rabbits	15 leukæmic patients	110	46	20	15	19
11 Rabbits	12 non-leukæmic patients	30	3	3	30	63
10 Rabbits	Leukæmic patient 'WOZ'	18	61	28	11	0
8 Rabbits	Leukæmic patient 'SOD'	11	36	0	27	36
Rabbit *B*	13 leukæmic patients	19	84	11	5	0
Rabbit *C*	9 leukæmic patients	10	0	20	40	40
Rabbit *B*	8 non-leukæmic patients	8	13	0	37	50
13 Guinea pigs	11 leukæmic patients	34	0	71	26	3
9 Guinea pigs	9 non-leukæmic patients	25	0	4	48	48
33 Non-leukæmic patients	17 leukæmic patients	36	0	0	0	100
17 Non-leukæmic patients	18 non-leukæmic patients	18	0	0	0	100

The rabbit sera gave early toxic reactions in 46 per cent of 110 tests on lymphocytes from 15 leukæmic patients (Table 1) and in 3 per cent of 30 tests on normal lymphocytes. The median 10 per cent survival time of non-leukæmic lymphocytes was 9·5 days when the cells were in human sera and 5·7 days in rabbit sera. Evidently, the rabbit sera were frequently rapidly toxic to leukæmic lymphocytes but usually had only a delayed toxic effect on normal lymphocytes.

The variability of the results is exemplified in Table 1 by the results on the cells of patients 'WOZ' and 'SOD' and on the sera of rabbits *B* and *C*. The complete data indicated that rabbits differed in the toxicity of their sera to leukæmic lymphocytes, that leukæmic patients differed in the sensitivity of their blood lymphocytes to the toxic sera, and that the sera of some rabbits differentiated between normal and leukæmic lymphocytes in a high percentage of tests.

Observations with an inverted phase microscope indicated that sensitive leukæmic lymphocytes in rabbit sera died by 'fixation', a process similar to that seen in lymphocytes irradiated with 10,000 r.[4]. In contrast, the normal lymphocytes in rabbit sera died by the development of intranuclear vacuoles and pyknosis, which has been studied by cinemicrography[5]. Normal granulocytes and erythrocytes, unlike normal lymphocytes, were sensitive to rabbit sera and lysed within an hour.

Early experiments with guinea pig sera gave the unexpected finding that leukæmic lymphocytes were frequently more sensitive to inactivated (56° C., 30 min.) than to fresh sera. The inactivated guinea pig sera gave a short (1–3 days) 10 per cent survival time in 71 per cent of tests with leukæmic lymphocytes and in only 4 per cent with normal cells.

The present results showed that, on the average, human leukæmic lymphocytes were sensitive to toxic factors in normal rabbit sera and in inactivated guinea pig sera. The rabbit sera usually killed the leukæmic lymphocytes in a few hours by 'fixation' and killed normal lymphocytes in a few days by intranuclear vacuolization. The rabbits differed in the toxicity of their sera and the leukæmic patients varied in the sensitivity of their lymphocytes to the toxic factors.

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Influence of Circulating Adrenocorticotrophin on the Pituitary Adrenocorticotrophic Response to Stress in the Adrenalectomized Rat

Removal of the adrenal glands results in a rise in the level of adrenocorticotrophic hormone in the blood of the rat. Cox and Hodges[1] reported that this increase did not occur until two weeks after adrenalectomy and that, after three to five weeks, there existed a remarkably high concentration of circulating hormone. These results were not inconsistent with the findings of Fortier[2], who showed that adrenalectomy caused a marked increase in the pituitary content of adrenocorticotrophic hormone after a similar time-lapse. It appears that there is an interval of at least two weeks before the rat pituitary gland adapts itself to secrete and maintain this enormously increased output of adrenocorticotrophic hormone in response to the absence of circulating corticoids. Therefore, it was of interest to compare the response to stress in adrenalectomized rats with low blood levels of corticotrophin with that in adrenalectomized animals with high blood levels of the hormone.

Adrenalectomized female Wistar rats were subjected to the stress of ether anæsthesia for 2½ min. at various times after the removal of their adrenal glands. The animals were decapitated, blood was collected from their trunks, injected intravenously into assay rats treated with hydrocortisone and its ability to produce depletion of adrenal ascorbic acid (which is a measure of its adrenocorticotrophic hormone content) determined, as described by Cox, Hodges and Vernikos[3]. The results were compared with those obtained using control adrenalectomized rats which were bled without being subjected to any previous stress. The results are summarized in Table 1.

It may be seen from Table 1 that exposure to ether vapour results in a marked increase in the circulating level of adrenocorticotrophin in the adrenalectomized rat. Furthermore, in contrast to the resting-level, the blood concentration of adrenocorticotrophic hormone after stress is independent of the time-lapse after adrenalectomy. The greatest increases occurred in the rats with the lowest initial levels of the hormone.

These results cannot be explained on the basis that the stores of corticotrophin in the pituitary gland become progressively exhausted after adrenalectomy, since several workers[2,4,5] have shown that removal of the adrenal glands results in a marked increase in the pituitary corticotrophin content. It appears more likely that the secretion of adrenocorticotrophin may be suppressed by a pre-existing high level of the hormone in the blood. The existence of such an 'auto feed-back' mechanism was also suggested by Kitay, Holub and Jailer[6], who found that the administration of exogenous corticotrophin to adrenalectomized rats prevented the usual fall in pituitary corticotrophin induced by stress. Thus, it appears that the circulating level of adrenocorticotrophic hormone may be yet another factor which may influence its release from the adenohypophysis.

Table 1. Adrenal Ascorbic Acid Depletions in Rats treated with Hydrocortisone after Intravenous Injections of Blood from Stressed and Non-stressed Adrenalectomized Rats (3 ml./100 gm. Body-weight)

Adrenal ascorbic acid level in saline-injected controls was 500 ± 10 mgm./100 gm. The figures in brackets indicate the number of animals injected

Time interval after adrenalectomy (days)	Mean ascorbic acid depletion (mgm./100 gm. adrenal tissue ± standard error)	
	Non-stressed	Stressed (ether 2½ min.)
5	−8 ± 13 (6)	126 ± 6 (12)
10	34 ± 8 (6)	146 ± 5 (12)
25	79 ± 8 (42)	139 ± 7 (6)
35	72 ± 12 (6)	155 ± 8 (6)

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Effect of some Lytic Agents on *Aerobacter aerogenes*

In the course of a study of the surface structures of *Aerobacter aerogenes* the action of lysozyme has been investigated. Preliminary experiments indicated that the enzyme produced no observable changes in the optical density of cell suspensions. In an attempt to promote enzymic attack the conditions were varied by altering the concentration of lysozyme, the time and temperature of incubation, the origin and age of the bacteria, and the *p*H of the suspensions. Cells were also incubated with lysozyme and various concentrations of sodium chloride, magnesium chloride, versene[1] and *tris*-buffer[2]. No lysis which could be attributed to the action of lysozyme was observed under any of these conditions. Microscopic studies showed the appearance, in solutions containing 20 per cent sucrose, of spherical bodies, which were probably similar to the protoplasts obtained by Weibull[3] from *B. megatherium*. These, however, did not constitute more than 1 per cent of the cell population, except in one case, when bacteria incubated overnight with a 2 per cent solution of lipase were subjected to the action of lysozyme in 20 per cent sucrose. About 5 per cent conversion to spheres was observed. In a test for the presence of lysozyme substrate in the cell walls of *Aerobacter aerogenes*, washed cells were suspended in saline at *p*H 3·6 and incubated for 1 hr. at 40° C. with 200 μgm./ml. of lysozyme[4]. The optical densities of the suspensions were measured before and after incubation. The *p*H was adjusted to 10·0 by the addition of sodium hydroxide and the optical density measured immediately. A comparison with the results of a similar experiment in the absence of the enzyme showed that there was some lysis due to lysozyme. The specific substrate is thus present in the cell walls of the organism, but it is doubtful whether the amount available to the enzyme is sufficient to permit extensive lysis.

Lederberg[5] described the preparation of protoplasts from cells of *Escherichia coli* by allowing them to divide

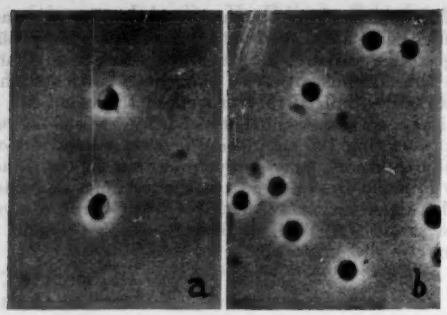

Fig. 1. Spherical bodies of *Aerobacter aerogenes* in 1·0 *M* sucrose (*a*) and in 0·2 *M* sucrose (*b*). (× 1,000)

in the presence of high concentrations of penicillin in a stabilizing medium. It was found that cells of *Aerobacter aerogenes* growing in a simple synthetic medium were lysed by 500 units/ml. of penicillin. However, cell division was suppressed by the high concentrations of sucrose or electrolyte necessary for the survival of any protoplasts formed. The organisms were able to grow and divide normally in 'Lab-lemco' broth containing 20 per cent sucrose. Accordingly, 200 ml. of an overnight broth culture was added to 600 ml. of broth containing sucrose (final concentration 0·6 *M*) and penicillin (1,000 units/ml.). After incubation for 3 hr. at 37° C. with gentle shaking, all the rapidly growing rod-shaped bacteria were converted to spheres. These were fragile to thermal, osmotic and mechanical shock. They could be harvested by centrifugation in the cold and resuspended in solutions of high osmotic pressure, for example, sucrose, glucose, sodium chloride or mixed phosphate buffers. By suspending the spheres in various solutions of sucrose at *p*H 6·8 and determining the concentrations which just prevented lysis at 25° C., their internal osmotic pressure was estimated at 5–6 atmospheres. The osmotic pressure of these bodies, which probably represents the mean osmotic pressure of the cells, is similar to that of *Escherichia coli*[6] and low compared with that of Gram-positive *Staphylococcus aureus* (25–30 atmospheres)[7].

There is some evidence that the spherical transformation undergone by rod-shaped bacteria exposed to the action of penicillin produces bodies the surface of which consists of material which can be differentiated from the cytoplasmic membrane. The spheres can undergo reversible plasmolysis when subjected alternately to high and low (5 atm.) external osmotic pressures in solutions of sucrose or sodium chloride. In concentrated solutions the protoplasm contracts away from the spherical shell surrounding the body. Addition of water causes it to expand until the sphere is filled completely (compare Figs. 1*a* and *b*). Evidently the protoplasmic membrane can become detached from the outermost material. These findings were confirmed by turbidity measurements. They are also in agreement with the observation made by Tomcsik[8], who found traces of cell-wall material on the spherical bodies obtained from *B. anthracis* treated with penicillin. It appears, therefore, that the term 'protoplasts' must be applied cautiously to such spherical bacterial bodies if it implies the complete absence of cell-wall material. Penicillin, while interrupting the biosynthesis of the main part of the cell wall, permits the formation of a fragmentary structure, which is unable to confer rigidity on the bacterial cell.

It is hoped to carry out electrophoretic studies on these bodies in an attempt to characterize their surface. Full details of these and other studies will be reported later.

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Release of Histamine from Mast Cells by Tissue Extracts

It is now established that the mast cells are an important source of histamine in the body[1]. Histamine can be released from these cells *in vivo* by the injection of certain substances, such as compound 48/80[2] and distilled water[3], but the mechanism of its release in pathological conditions, such as anaphylaxis and tissue injury, has not yet been satisfactorily explained. This problem has been investigated by exposing isolated mast cells from the rat to tissue extracts. It was found that injured nucleated cells produce a factor which liberates histamine from mast cells with histological changes in the mast cells varying from cellular swelling to cellular disintegration.

The mast-cell suspensions were prepared by injecting 40 ml. of physiological saline solution into the peritoneal cavity of the rat and aspirating the fluid after approximately 30 sec. Cellular elements were concentrated by centrifuging the aspirate. The mast cells were separated by layering the cellular concentrate on top of a 4-cm. column of 50 per cent albumin solution in a Wintrobe hæmatocrit tube and centrifuging the tube at 250*g* for 5 min. The mast cells were recovered from the bottom of the tube and were found to be almost free of other types of cells, which remained above the albumin layer.

Histamine was measured by the colorimetric method of Lowry *et al.*[4]. It was found that histamine was released from these mast cell preparations by mechanical grinding, by the addition of distilled water, and by the addition of compound 48/80 (20 μgm./ml.) protamine (10 μgm./ml.), *d*-tubocurarine (1 mgm./ml.) and peptone (1 mgm./ml.). Approximately 40 mgm. of histamine was obtained per million mast cells.

Histamine was also released from mast cells by saline extracts of spleen, kidney, liver and lung tissue from the rat and the rabbit. Histamine was released by saline extracts of pigeon red cells and of

human white cells, but not by extracts of human or rat red cells. The extracts were prepared by grinding the tissue in an equal volume of physiological saline solution and incubating the homogenate at 37° C. for 30 min. Cell debris was removed by centrifugation. The active principle in splenic extract was not destroyed by boiling and was not removed by centrifugation at 28,000*g* for 30 min. It was stable in acid (0·25 *N* perchloric acid for 15 min. at 90° C.) and in alkaline solution (0·3 *N* potassium hydroxide for 24 hr. at 20° C.). Dialysis of the neutralized acid-treated extract against distilled water produced a precipitate which contained the active principle and gave a positive Sakaguchi reaction for arginine, a positive biuret reaction, and a negative reaction for carbohydrate. These findings suggest that the active principle is a long-chain polypeptide containing arginine.

Preliminary (unpublished) experiments have been performed in collaboration with K. Ellem, in which mouse ascites tumour cells and fresh antisera prepared in rabbits against the tumour cells were incubated for 30 min. at 37° C. A substance appeared in the serum which caused disintegration of mast cells with liberation of histamine. When non-immune rabbit serum was substituted for the antiserum, histamine was not released. In association with W. R. Sobey a similar phenomenon was observed with human white cells and fresh anti-human white cell serum prepared in rabbits (unpublished).

These experiments *in vitro* with mast cells suggest that injured nucleated cells produce a factor which leads to the disintegration of mast cells and the release of histamine. The active principle derived from injured cells appears to be an arginine containing polypeptide. The same mechanism may be responsible for the release of histamine when cells are injured *in vivo*.

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Inherited Hypertension in Rats

During the past eight years a colony of hypertensive rats has been developed by breeding from rats with over-average tail blood pressures. The mean blood pressure of the Otago rat colony from which rats were selected for breeding is of the order of 124 mm. mercury for bucks, 116 mm. mercury for does.

We have developed several families with a high incidence of hypertension by selective cross-breeding, and in three strains (*A*, *B* and *C*) by intensive inbreeding, using brother–sister mating. Selective cross-breeding has produced higher pressures than inbreeding (Fig. 1).

Comparison of blood-pressure levels of unanæsthetized rats, gently restrained, and of the same rats under light ether anæsthesia revealed no important difference in the means. The average blood pressures

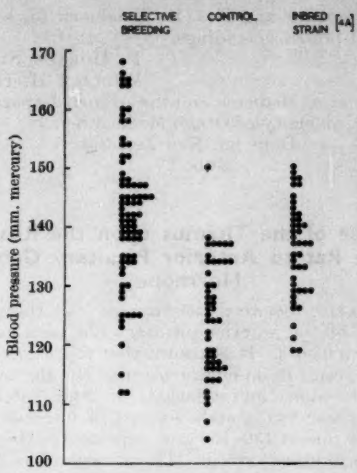

Fig. 1. Blood pressures of rats with inherited hypertension as compared with control rats. Each dot represents the average of several readings in a rat

of 70 rats, some hypertensive and some controls, without anæsthesia was 131·9 and under light ether anæsthesia was 129·8 mm. mercury. Direct recording of pressures with a tube in a femoral artery disclosed great variability in the blood pressure of the rat, both under anæsthesia and unanæsthetized. While there is a close relationship between tail pressures and direct pressure readings from a femoral artery, the fluctuations of the blood pressure-level make it necessary to obtain readings on several occasions for each rat in order to obtain an average value.

The general impression is that we can expect at least 30 per cent of buck rats in the Otago hypertensive colony to have tail pressures exceeding 140 mm. mercury. In strains cross-bred selectively we may reasonably expect more than 10 per cent of bucks to have pressures exceeding 150 mm. mercury and occasional bucks to have pressures of 160–170 mm. mercury.

The average pressure of 50 control buck rats measured recently was 124·14 (standard deviation 10·64) mm. mercury; of 43 buck rats of strain *A*, 135·81 (standard deviation 8·20). The difference of the means is about five times the standard error of difference (1·96) and highly significant. The mean blood pressure of 76 selectively cross-bred buck rats was 141·95 (standard deviation 12·53) mm. mercury, which is 17·81 mm. mercury greater than the mean for control rats. The difference of the means is in this case more than eight times the standard error of difference (2·08).

The selectively bred does have a mean pressure of 135·37 and the fourth generation of the *A* inbred strain 129·91, as against 116·33 mm. mercury for the Otago control colony. The differences of the means are in each case more than five times the standard errors of difference and therefore highly significant.

Some results will be published later by Dr. Arthur Veale relating the blood pressure as estimated by the tail method in the rat to the width of cuff, use of ether anæsthetic, absence of an anæsthetic, position of the cuff and recorder on the tail, direct warming of the tail, use of a thermostatically controlled room, etc. We are indebted to Mr. C. Drake,

Dr. I. Eryetishir and Mrs. R. Shacklock for some of the blood-pressure readings.

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Influence of the Thymus upon the Reaction of the Rat to Anterior Pituitary Growth Hormone

A relation between the thymus and the growth hormone of the anterior pituitary has been assumed by many authors. It is known that injections of this hormone result in an enlargement of the thymus[1]. A more thorough investigation of the interaction between these two glands seemed of interest.

80 male rats of 130–160 gm. were used. They came from a local inbred strain. The animals were divided into the following groups: (*A*) 20 animals were hypophysectomized by the method of Smith, as described by Farris and Griffith[2]; (*B*) 60 animals were thymectomized by the method of Segaloff[3] and hypophysectomized 8 days later.

Following hypophysectomy, the animals were kept at a constant temperature of 29° C. Growth was recorded for 15 days by daily weighing to the nearest 0·5 gm. The hormone injections were begun the 16th day after hypophysectomy: (*a*) the 20 hypophysectomized animals received daily injections of a commercial growth hormone preparation at daily doses of 0·5, 1·0, 2·0, 3·0 and 4·0 Evans units (4 animals at each dose); (*b*) 20 animals from the thymectomized and hypophysectomized group received the same injections; (*c*) 20 thymectomized and hypophysectomized animals were injected daily with the thymus preparation of Bezssonoff and Comsa[3] at daily doses of 5, 10, 20, 35 and 50 guinea pig units[4] (4 animals at each dose); (*d*) 20 thymectomized and hypophysectomized animals were injected similarly with the same thymus preparation. The thymus injection was followed immediately by an injection of growth hormone at a dose of 2·0 Evans units in all animals. The injections were continued for 15 days with daily weighing.

During the 15 days between hypophysectomy and the first hormone injection, weight loss was constant in all animals. It amounted to about 12 gm. in both groups (1 and 2).

The results during the hormone-injection period are shown in Tables 1 and 2. It can be seen that: (1) in group *A* the growth-rate under the influence of the same dose of growth hormone was significantly smaller than in group *B*. (2) Thymus extract injections alone had no real influence upon the growth-rate of group 2 animals. The loss in weight observed in the preperiod ceased following the thymus injections, but no proper growth was observed. (3) The simultaneous injection of thymus extract and 2·0 Evans units of growth hormone resulted in a significant enhancement of the influence growth hormone upon the growth-rate.

Table 1. Influence of Daily Injections of Anterior Pituitary Growth Hormone upon the Growth-Rate of the Rat

Hormone daily dose (Evans units)	Weight difference after 15 days of daily injections (arithmetical average of three animals)	
	A Hypophysectomized	*B* Thymectomized and hypophysectomized
0 (preperiod)	−12·8 ± 3·0	−12·0 ± 1·0
0·5	0	−4 ± 1·0
1·0	15·0 ± 3·8	6·5 ± 2·2
2·0	30·0 ± 5·1	11·5 ± 2·0
3·0	41·0 ± 7·2	12·0 ± 3·0
4·0	56·0 ± 5·0	17·0 ± 3·0

Table 2. Influence of Daily Injections of Thymus Extract upon the Growth-Rate of Thymectomized and Hypophysectomized Rats

Thymus daily dose (units per rat)	Weight difference after 15 days of daily injections (gm., arithmetical average of three animals)	
	C Injected with thymus only	*D* Injected with thymus and 2·0 units of growth hormone
0	−12·0 ± 1·0	+11·5 ± 2·0
5	−8 ± 0·8	+30·0 ± 4·0
10	0	+45·0 ± 7·0
20	−0·5 ± 0·2	+58·0 ± 6·0
35	−3·0 ± 0·5	+64·0 ± 6·4
50	−1·5 ± 0·6	+80·0 ± 4·8

The thymus is a significant factor in the growth effect of anterior pituitary growth hormone.

We are indebted to Miss L. Fehres for technical advice, and to Messrs. Choay (Paris) for growth hormone.

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Synapse Discharge by Single Fibre in Mammalian Visual System

Following a single brief electrical shock applied to the optic nerve in the cat there is, from the lowest levels of stimulation, an approximately linear relation between the amplitudes of the pre- and post-synaptic responses recorded in the lateral geniculate body[1]. This was taken to indicate that only a small number of optic tract fibres are needed to discharge a geniculate cell.

In the course of recording extracellularly from single units in the visual pathways of the Dial-anæsthetized cat we have obtained further evidence in favour of this view. In the case of at least two geniculate cells that we have been able to study in some detail we conclude that they were each being discharged by a single optic nerve fibre. The evidence for this is illustrated in Figs. 1 and 2. Records *a* and *b* in Fig. 1 are representative of a large number obtained in the vicinity of a single geniculate cell in response to a stimulus of constant strength applied to the ipsilateral optic nerve; (*a*) consists of three and (*b*) of many superimposed oscillograph traces. It is clear that only three types of response are obtained: (1) a spike potential, (2) a synaptic potential (that is, an excitatory postsynaptic potential[2] recorded extracellularly) and (3) neither of these.

Our evidence that (2) is a synaptic potential is as follows: it always precedes the spike potential as

a step on the rising phase; it has the time course of synaptic potentials obtained elsewhere[3]; it sums with a second such potential (Fig 1*e*); it shows progressive depression during a train of evoked responses (Fig. 1*e*), in this respect resembling the end-plate potential at the mammalian neuromuscular junction[4]. Finally, in recording from other single geniculate cells, we have found that *d*-lysergic acid diethylamide (*LSD* 25) abolishes the spike potential, leaving a potential which closely resembles response (2) of Fig. 1. A similar result can also be obtained by placing the test shock in the wake of a brief but rapid train of conditioning stimuli applied to the optic nerve. Fig. 3 shows the effect of a critical blocking dose of *d*-lysergic acid diethylamide, the response prior to the application of the drug being similar to (*b*). During the action of the drug the response varied between the two forms (*a*) and (*b*) of Fig. 3. A further dose of the drug abolished the spike potential and reduced the amplitude of response (*a*) without altering its time course. It has previously been concluded[5] that *d*-lysergic acid diethylamide blocks transmission in the lateral geniculate body by reducing the postsynaptic response to a non-propagating postsynaptic potential.

Our interpretation of the records in Fig. 1 is that the stimulus is critical for a single optic nerve fibre. If the fibre is not excited we obtain response (3), the electrical field response due to other activated fibres and cells; if the optic nerve fibre fires we obtain either response (1) or response (2), that is to say, the synaptic potential is about threshold for the discharge of the geniculate cell. There is no variation in response (2) beyond what is to be expected in the baseline itself under these recording conditions. The spike potential in response (1) arises from the synaptic potential with no detectable increase in amplitude of the latter. If another optic nerve fibre was needed for the cell to discharge then one ought to see an increment in the synaptic potential before the spike arose, and one ought also to see occasionally this second synaptic potential alone; neither was seen.

The spike potential does have another step on its rising phase (in the reproduced photographs clearly visible only in Fig. 1, where it is indicated by arrow). It is unlikely that this step represents a second synaptic potential summing with the first because it is always intermediate between the synaptic potential and the full spike and has a faster rising phase; if it were a synaptic potential one should occasionally see it either alone or initiating the full spike potential of its own accord.

It is not yet known to what degree synaptic activity in different parts of one cell can be recorded by an extracellular electrode. The possibility, therefore, of an unrecorded additional synaptic potential is difficult to exclude. However, in this case, the spike potential was never initiated except after the synaptic potential. This fact excludes the possibility of any other synaptic potential capable of firing the cell, and we feel that it is unlikely that synaptic potentials of any significance have gone unrecorded.

The records in Figs. 1 and 3 were obtained using glass capillary microelectrodes filled with 3 *M* potassium chloride, having a d.c. resistance of about 5 megohms. Those in Fig. 2 were obtained with a fine steel electrode insulated to the tip; records *a*, *b* and *c* are varying responses to a stimulus of constant strength. The remarks made in connexion with Fig. 1 apply also to this cell.

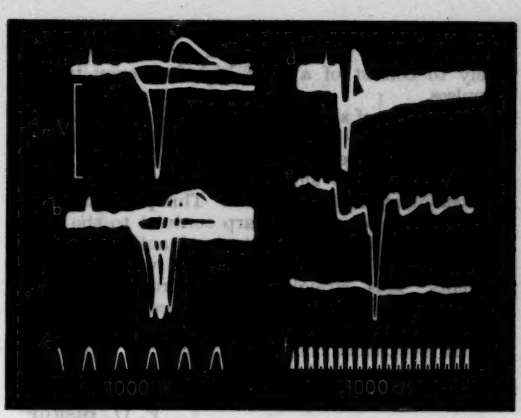

Fig. 1. Extracellular records from single lateral geniculate cell to electrical stimulation of ipsilateral optic nerve. (*a*) and (*b*) Stimulus of constant strength; (*a*) three and (*b*) many successive traces superimposed. Arrow indicates step on rising phase of spike potential. (*d*) Similar series later in the experiment. (*e*) Responses to a train of five stimuli at 300/sec. Time calibration (*c*) applies to (*a*) and (*b*), calibration (*f*) to (*d*) and (*e*). Voltage calibration applies to all records. Negativity upwards in all figures. Spikes truncated in (*d*) and (*e*)

Responses essentially the same as those depicted ing Figs. 1 and 2 have been described by Freygang[6], who also regards response (2) as a synaptic potential. It is not proposed here to enter into the questions of the polarity of the response nor of the exact site of origin of its various components. These have been discussed by Freygang[6].

Our results do not mean that geniculate cells are not also innervated by optic fibres which on their own are incapable of discharging an impulse in the postsynaptic element. We have occasionally observed synaptic potentials which could be graded to some extent by varying the strength of afferent stimulation (cf. Freygang[6]), and the existence of a subliminal fringe around geniculate cells is well established from other studies[7]. These results, however, indicate the existence of 'single-fibre straight-through' pathways in the lateral geniculate nucleus. Although, in the cases described here, the synaptic potential is of critical strength for impulse propagation, certainty would be assured in the unanæsthetized animal and

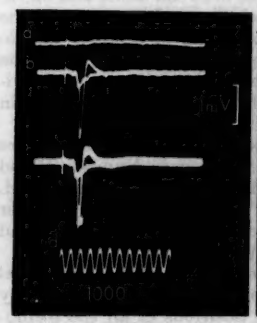

Fig. 2

Fig. 3

Fig. 2. (*a*) (*b*) and (*c*) Extracellular records from single lateral geniculate cell following electrical stimulus of constant strength to contralateral optic nerve. (*a*) No response, (*b*) three, (*c*) several successive traces superimposed. Voltage and time calibrations apply to all records. Spikes in (*c*) retouched

Fig. 3. Effect of *d*-lysergic acid diethylamide on response of single lateral geniculate cell to electrical stimulation of ipsilateral optic nerve. During action of drug response varied between forms (*a*) and (*b*). Before application of drug response similar to (*b*). Voltage and time calibrations apply to both records

especially in response to the physiological stimulus of light, since the response in an optic fibre is then likely to consist of a brief high-frequency train of impulses.

This ability of single optic nerve fibres to discharge geniculate cells is almost certainly made possible by the fact that the preterminals branch very extensively[8], so that a single fibre may apply a large number of terminals to a cell. This property of the geniculate synapse is in sharp contrast to the spinal motoneuron, which integrates and acts as the final common path for impulses from a wide variety of afferents. This 'straight-through' property of many geniculate cells by no means excludes the possibility of important modifications in the signal taking place at this level, since changes can occur in the pattern of the repetitive discharge which frequently follows a single afferent volley[9].

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Larval and Adult Hæmoglobins of the Cyclostome *Petromyzon planeri*

Systematic investigations of the electrophoretic behaviour of hæmoglobins of various vertebrates have so far been carried out mainly on the higher forms. We have started to investigate the electrophoretic behaviour of hæmoglobin of the cyclostome *Petromyzon planeri* in the adult or in the ammocoetes stage.

Paper electrophoresis did not permit the detection of any consistent differences between adult and larval blood, which by this technique showed the same electrophoretic pattern consisting of a single hæmoglobin band migrating toward the anode. On the other hand, starch-gel electrophoresis provided evidence for the presence of two distinct hæmoglobin bands with different mobility at each stage.

Adult and ammocoetes of *Petromyzon planeri* were collected from the River Sarno near Naples. Blood from 32 adults and 44 ammocoetes has been examined. Blood was collected, by decapitation, in Alsever solution. Erythrocytes were washed repeatedly, and then hæmolysed with distilled water.

Electrophoresis was performed with starch gel using the discontinuous buffer system described by Poulik[1]. Fig. 1 shows the positions of all the hæmoglobin components in the adult and ammocoetes stages. Adult blood has two hæmoglobin bands (I and II). Hæmoglobin I migrates more rapidly and is present in much smaller amounts than II. Larval blood also has two distinct hæmoglobin bands (I′ and II′), which are apparently present in the same concentrations. They migrate more rapidly than the adult ones.

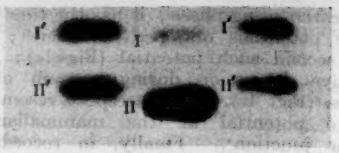

Fig. 1

Apart from the difference in the electrophoretic mobility between adults and larvæ, no other differences were detected among animals of the same stage and in particular no animals with only a single band were found in Nature.

It is conceivable, therefore, that the double-band pattern obtained only by gel electrophoresis might be the result of some kind of degradation of the hæmoglobin molecule due to the supporting medium itself.

Further researches are in progress to clarify this aspect of the problem, bearing in mind what is known for mammalian hæmoglobins[2–4].

Nevertheless we think that the results given above allow the conclusion that in these lower vertebrates the electrophoretic behaviour of hæmoglobin in the larval stage is different from that of the adult stage. Although this may be obviously interpreted as to be due to the presence of some fœtal-like hæmoglobin in the larvæ, it must be noted that unlike the mammalian fœtal hæmoglobin, here the larval hæmoglobin migrates faster than the adult one.

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Respiration and Radiosensitivity of Broad Bean Roots

In the root-tips of the broad bean, *Vicia faba*, potassium cyanide, hydrogen sulphide and cupferron (the ammonium salt of nitrosophenylhydroxylamine) increase the frequency of chromosome aberrations produced by a given dose of X-rays at very low oxygen pressures[1–3]. In the case of cupferron at least, the effect on radiosensitivity seems to be a result of its inhibitory action on the respiration of bean root tips[3]. The fact that oxygen enhances the radiosensitivity of the root-tip chromosomes of *Vicia faba* has been known since the studies of Thoday and Read[4]. When the roots are respiring normally an oxygen effect does not become evident until the concentration of oxygen in the gas phase exceeds 3 per cent, apparently because at lower concentrations oxygen is removed by respiration in the outer cells of the root, so that completely anaerobic conditions prevail in the central parts. When respiration is inhibited, oxygen is able to diffuse into the cells of the whole meristem, even in cases when the oxygen concentration outside the root is very low. That this is the correct explanation of the increased frequency of aberrations obtained in the presence of cupferron is indicated by the fact that cupferron has no marked influence on radiosensitivity in the complete absence of oxygen[3].

Unlike cyanide and sulphide, cupferron has not been known previously as a respiratory inhibitor. In experiments performed with the aid of the Warburg manometric technique, it has now been found that the respiration of excised bean root tips is markedly inhibited by cupferron. The effect is influenced by the *p*H of the solution and also, to some extent, by the oxygen pressure. A low *p*H and a low oxygen tension favour the inhibitory effect of cupferron. At an oxygen pressure of 2·8 per cent and a *p*H of 5·5, the inhibition produced by 400 μmoles/l. cupferron varies between 70 and 100 per cent. As appears from Table 1, there is a correlation between the effect of cupferron on radiosensitivity and its effect on respiration.

By using cupferron as a respiratory inhibitor, it has been possible to obtain a relation between oxygen pressure and the radiosensitivity of the root-tip chromosomes of *Vicia*, which approaches the true one more closely than that obtained when respiring root-tips are used. This relation is expressed graphically in Fig. 1. As a comparison, the relation between oxygen pressure and effect was also determined in the absence of cupferron and the resulting curve is included in Fig. 1.

Since preliminary X-ray experiments indicated that no further increase of radiosensitivity is obtained by decreasing the *p*H below 5·8 or by increasing the cupferron concentration above 400 μmoles/l., the *p*H was kept between 5·8 and 6·0 in subsequent experiments and the concentration of cupferron was 400 μmoles/l. Oxygen–nitrogen mixtures containing 0·1, 1·0, 2·8, 5·4 and 10·1 per cent oxygen were supplied by the Swedish Gasaccumulator Company. The other oxygen concentrations used in the experiments were obtained by mixing gases with known concentrations of oxygen in appropriate proportions by the aid of flow-meters. Oxygen-free nitrogen was obtained by passing tank nitrogen over active copper in a Meyer–Ronge column[5]. The gases were introduced into the 'Lucite' vessels used for the treatments through fritted glass filters. The beans were kept under anaerobic conditions for 10 min. before an oxygen-free solution of the inhibitor was introduced. The required oxygen–nitrogen mixture was then bubbled through the solution for 25 min. before the irradiation and the bubbling was continued during the irradiation itself. The X-rays were generated in a Müller therapy tube type 71780, operating at 180 kV., 5 m.amp. and filtered by 1 mm. of aluminium. The dose was 100 r. and the dose-rate 20 r./min. The beans were fixed 18 hr. after the irradiation. Permanent Feulgen-squashes were prepared as described previously[6].

It appears from Fig. 1 that, in the presence of cupferron, 1·3 μmoles/l. oxygen is enough to increase the radiosensitivity. The number of isolocus breaks and exchanges obtained per 100 cells under anaerobic conditions is more than doubled at an oxygen concentration of 3·9 μmoles/l., and at 36 μmoles/l. the maximum radiosensitivity seems to have been reached. In the absence of cupferron, no significant increase of radiosensitivity was obtained at oxygen concentrations below 65 μmoles/l. Fig. 1 also shows that the effect is considerably greater in the presence than in the absence of cupferron at high oxygen tensions. The explanation given above for the effect of cupferron on radiosensitivity does not seem to be applicable to the increased effect obtained at high oxygen tensions in the presence of cupferron. The latter may possibly be due to the radiomimetic effect of cupferron[7], although ½-hr. treatments with 400 μmoles/l. cupferron alone as a rule do not produce any chromosome aberrations.

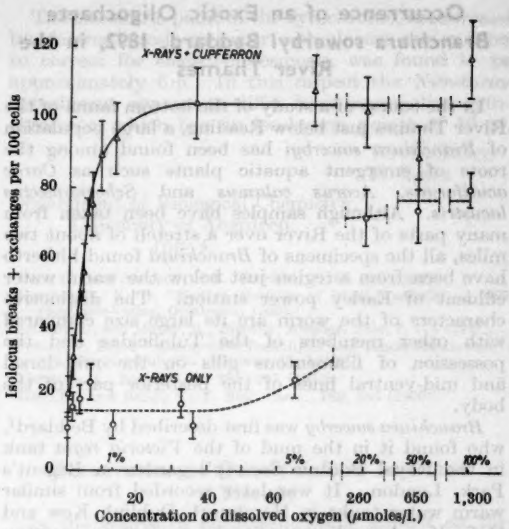

Fig. 1. The relation between the concentration of dissolved oxygen and the frequencies of chromosome aberrations induced by 100 r. of X-rays in the presence and the absence of cupferron

Table 1. The Effect of 200 μmoles/l. Cupferron on the Frequency of X-Ray-induced Chromosome Aberrations and on Oxygen Consumption at *p*H 5·5 and 7·3

	X-ray experiments				Respiration experiments	
*p*H	Concentration of oxygen in the gas phase (per cent)	Abnormal metaphases (per cent)	Aberrations/100 cells: Isolocus breaks	Aberrations/100 cells: Exchanges	Concentration of oxygen in the gas phase (per cent)	Inhibition of oxygen consumption (per cent)
5·5	1·0	68	52	50	2·8	83
7·3	1·0	22	9	15	2·8	11

The X-ray–cupferron curve in Fig. 1 is very similar to the curve obtained by Howard-Flanders and Alper for the radiosensitivity of the bacterium *Shigella flexneri* at different oxygen tensions[8]. In another series of experiments, the constants m ("maximum enhancement ratio") and K ("that oxygen concentration which will give $(m + 1)/2$ times the nitrogen radiosensitivity") of Howard-Flanders and Alper[8] were established. The value of K obtained in the presence of cupferron at 22° C., 4·2 ± 0·9 μmoles/l., is also very close to that obtained by Howard-Flanders and Alper for *Shig. flexneri*. In the absence of cupferron the value of K was found to be about 132 μmoles/l. Thus, when respiration is inhibited, the radiosensitivity of the root-tip chromosomes of *Vicia faba* is affected by a concentration of oxygen which is about thirty times lower than that effective in the absence of the inhibitor.

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Occurrence of an Exotic Oligochaete *Branchiura sowerbyi* Beddard, 1892, in the River Thames

In the course of a study of the bottom fauna of the River Thames just below Reading, a large population of *Branchiura sowerbyi* has been found among the roots of emergent aquatic plants such as *Carex acutiformis*, *Acorus calamus* and *Schoenoplectus lacustris*. Although samples have been taken from many parts of the River over a stretch of about two miles, all the specimens of *Branchiura* found hitherto have been from a region just below the warm water effluent of Earley power station. The distinctive characters of the worm are its large size compared with other members of the Tubificidae and the possession of filamentous gills on the mid-dorsal and mid-ventral lines of the posterior part of the body.

Branchiura sowerby was first described by Beddard[1], who found it in the mud of the *Victoria regia* tank in the Royal Botanic Society's garden in Regent's Park, London. It was later recorded from similar warm water tanks in Hamburg[2], Dublin[3], Kew and Oxford[4]. According to Stephenson[5], it is common in India, China, Japan and Java, and occurs in the Rhone and elsewhere in southern France, possibly as a result of being introduced with plants imported into gardens on the banks of the rivers. The first person to find *Branchiura* in a natural habitat in Britain was Dr. P. M. Jenkin, of the Zoology Department, University of Bristol. In a recent private communication, she described her findings as follows: "A small colony has persisted for five years on the Kennet and Avon Canal. The species has now appeared for the first time in the Bradford River Avon, at a spot where collections have been made for twenty years. Temperature records stored in the University of Bristol Zoological Department show no indication of warm effluents near either of these sites. The canal is so shallow that it must freeze in the winter. The worms live in organic mud under 4–6 inches of water. They survive well in the laboratory in tubes containing tap water and some mud from their natural environment."

The population of the worms in the Thames is not a small one, as it is estimated from quantitative samples that many thousands are present. They are obviously breeding very freely, and it seems likely that the warm water effluent of the power station (maximum temperature observed, 25° C.) is a more favourable habitat than the unheated waters of the Kennet and Avon Canal. On the other hand, the fact that the worm has appeared in a second unheated habitat suggests that it has become acclimatized sufficiently to be able to increase its numbers in a normal river. That being so, it may well spread through the river and canal system of Britain, and I would be most interested to hear of further records.

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Histochemical Study of the Nasal (Supra-Orbital) Gland of the Duck

The recent demonstration by Schmidt-Nielsen and co-workers[1,2] that in marine birds the nasal (supra-orbital) glands can, under conditions of salt loading, excrete a strongly hypertonic solution of sodium chloride has brought added significance to a study of the comparative cytology and histochemistry of these glands in progress in this department.

In the domestic duck, which has well-developed nasal glands, no histological evidence has been found to support either of the two earlier views on the nature of the secretion of these glands. One widely held opinion has been that the glands elaborate a 'serous' secretion. In the duck gland, cytoplasmic ribonucleic acid, assessed by affinity for toluidine blue and for pyronin, is notably scarce; the secretory elements are, indeed, conspicuously eosinophilic. Moreover, 'zymogen' granules are absent. The other view[3], that the gland produces a slimy (? mucous) secretion, also has no histochemical support. No mucus-secreting elements can be demonstrated by the periodic acid – Schiff technique, or with Alcian blue staining, and there is no metachromatic staining with toluidine blue.

The positive findings of the present study are (1) an extraordinary abundance of mitochondria, which appear almost to fill the cytoplasm of the secretory cells; (2) a moderate content of lipoid which is unstained by sudan III and IV, well stained by sudan black and gives a positive reaction to Baker's acid hæmatein test for phospholipids (pyridine extracted controls give a negative reaction: it seems likely that the phospholipids are mainly mitochondrial); (3) a very abundant blood supply; and (4) moderate amounts of alkaline phosphatase (3 hr. incubation, Gomori technique) in the nuclei, and minimal amounts in the cytoplasm, of cells at the extreme periphery of each gland lobule.

In their eosinophilia and high mitochondrial content, the secretory elements of the nasal gland of the duck are strongly reminiscent of the oxyntic cells of the gastric glands of mammals and of the chloride-excreting cells of the gill of euryhaline fishes, both of which are usually thought to be involved in active secretion of chloride ions.

Preliminary experiments indicate that the nasal glands of the duck are normally inactive, but that under conditions of salt loading they secrete chloride at concentrations of up to 640 m.equiv./litre—a level comparable with that reported for the cormorant[1] and the penguin[2].

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Cytochromes of *Nitrobacter*

A cytochrome system appears to be involved in the oxidation of nitrite by *Nitrobacter* since, at normal temperatures, three cytochrome bands may be seen in suspensions of the cells in the presence of nitrite, but not in its absence. These are two sharp bands at 521 mμ and at 550 mμ and a more diffuse, less-intense band at about 589 mμ [1].

We have now obtained further indications of involvement of cytochromes in nitrite oxidation since the oxidation has been found to be inhibited by carbon monoxide and by cyanide. We have therefore carried out a more detailed examination of the cytochromes of *Nitrobacter*.

In the presence of aqueous glycerol (50 per cent v/v), at the temperature of liquid air[2], the band at 589 mμ was resolved into two separate bands at 598 mμ and at 580 mμ. The former band probably represents that of the cytochrome *a* component of the cells.

In order to determine the distribution of the cytochromes *Nitrobacter* cells were crushed in the Hughes press and whole cells removed by centrifugation at 4,000*g*. The cell-free suspension thus obtained was separated into an insoluble fraction and a cell extract by centrifugation at 15,000*g*. The insoluble fraction, with dithionite, showed all the bands present in the whole cells, whereas the extract contained only the cytochrome with bands at 521 mμ and 550 mμ.

When the cells were disintegrated with the Nickle shaker and fractionated by the method of Salton and Horne[3], a preparation was obtained which, by electron microscopy, was shown to consist of cell walls. The preparation was deep red in colour and appeared to contain all the cytochromes present in the whole cells; the extract of the cells, however, as in the case of the extract of cells crushed in the Hughes press, contained only the cytochrome with bands at 521 mμ and 550 mμ.

One of the cytochromes present in the insoluble fraction of the cells crushed in the Hughes press appears to have cytochrome oxidase activity. The soluble cytochrome of *Nitrobacter* is not autoxidizable, but addition of a suspension of the insoluble fraction to the cell extract renders this cytochrome oxidizable by air; the insoluble fraction also catalyses the oxidation of reduced mammalian cytochrome *c* by air. Cytochrome oxidase activity in the insoluble fraction could also be demonstrated manometrically with ascorbate[4]; the uptake of oxygen by this fraction in the presence of ascorbate was increased by the addition of either the soluble fraction of the cells or by mammalian cytochrome *c*. This observation indicates that the insoluble fraction is relatively deficient in *c*-type cytochromes (probably the cytochrome with bands at 521 mμ and 550 mμ) and that the cytochrome oxidase of the insoluble fraction is capable of coupling with mammalian cytochrome *c*.

The similarity between the 550 mμ cytochrome and mammalian cytochrome *c* was further demonstrated by examination of an extract from crushed cells partially purified by ethanol fractionation at 0° C.

The spectrum of the cytochrome was found[5] to be similar to that of mammalian cytochrome *c*, with the main bands in the reduced form at 550 mμ, 521 mμ and 416 mμ. The spectrum of the reduced pyridine hæmochromogen of the cytochrome was identical with that of mammalian cytochrome *c*.

The E'_0 of the cytochrome was determined by titration with ferrocyanide in the presence of ferricyanide in an atmosphere of nitrogen. It was found to be in the region of 0·25 V. at *p*H 7·5 and 25° C. The reduction of the cytochrome appeared to involve a single electron transfer since the slope of the graph of log ferrocyanide/ferricyanide against log reduced cytochrome/oxidized cytochrome was 45° C.

The isoelectric point of the cytochrome, determined by electrophoresis on paper with glucose as a marker to correct for electro-endosmosis, was found to be approximately 6·5. In this respect the *Nitrobacter* cytochrome appears to differ from mammalian cytochrome *c*, which has an isoelectric point[6] of 10·65.

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Occurrence of Gentisic Acid in Plant Tissues

In the course of chromatographic studies on the phenolic constituents of *Theobroma cacao* L., an ultra-violet fluorescent compound (compound *A*), possessing an R_F value of 0·28 in benzene/acetic acid/water, was shown to be widely distributed throughout the tissues of the plant[1]. This compound has now been shown to possess chromatographic and ultra-violet fluorescence properties identical with those of gentisic acid (2 : 5 dihydroxy benzoic acid). Chromatographic examination of a large number of leaf hydrolysates has revealed that gentisic acid is widely distributed in the plant kingdom, and it would appear to be one of the most commonly occurring aromatic acids of plant tissue. This acid, although usually present in very small or trace quantities, can readily be detected by the chromatographic technique described. The presence of other fluorescent aromatic acids, namely, caffeic, ferulic and sinapic acids in leaf hydrolysates has recently been reported[2].

Although a good separation of compound *A* from the other fluorescent constituents of cacao leaf hydrolysates is effected by the use of the benzene/acetic acid/water system[3], the use of 20 per cent aqueous potassium chloride; *iso*propyl alcohol/ammonia/water[4] or chloroform/acetic acid/water[5] is rendered difficult by the fact that certain flavonoid and phenolic residues present in cacao extracts tend to conceal the gentisic acid spot. A preliminary separation was therefore carried out, the ethereal extract of the hydrolysate, prepared by the standard procedure[1] being applied to the chromatogram in a continuous band. After development with benzene/acetic acid/water the appropriate fluorescent band was cut out and eluted with hot methanol. The methanolic extract thus obtained was run against an authentic standard of gentisic acid (Delta Chemical Works, Inc.) in each of the four solvent systems shown in Table 1. In all cases the R_F values of compound *A* were identical with those of gentisic acid. The fluorescence of compound *A* was also observed to be identical with that of gentisic acid, namely, a bright blue, which turned green-white on exposure to ammonia fumes. It was noted that in both cases the greening with ammonia was of a much more transient character than is observed with the chlorogenic acids, the colour reverting to the

Table 1. Chromatographic Comparison of Gentisic Acid and Compound *A* isolated from Leaf Tissue of *T. cacao* L.

	Gentisic acid R_F	Compound *A* R_F
Benzene/acetic acid/water (60 : 70 : 30)	0·28	0·28
20 per cent aqueous potassium chloride	0·55	0·55
*iso*Propyl alcohol/ammonia/water (8 : 1 : 1)	0·71	0·71
Chloroform/acetic acid/water (2 : 1 : 1)	0·30	0·30

original bright blue immediately on removal from the ammonia.

Further confirmation of the identity of compound *A* with gentisic acid was obtained by spraying the chromatogram with an aqueous solution of ammonia (specific gravity 0·910) and immediately examining in ultra-violet light. Under these conditions the gentisic acid spot and that of compound *A* showed a characteristic yellow fluorescence. This change in the colour of the fluorescence from blue to yellow after spraying with ammonia was found to be highly specific for gentisic acid.

The procedure adopted for the routine screening of leaf tissue for gentisic acid was as follows: 2 gm. of fresh, finely shredded leaf tissue were digested with 10 ml. of 20 per cent aqueous hydrochloric acid (v/v) for 20 min. on a boiling water bath. After cooling and filtering, the filtrate was extracted with 2 ml. of diethyl ether and 0·1 ml. of this was applied with a micropipette to Whatman No. 4 chromatography paper, which was formed into a cylinder and developed with benzene/acetic acid/water[3]. The dried chromatograms were then examined in ultra-violet light and the gentisic acid spots located by their blue fluorescence. Confirmation of their identity was obtained by the use of the ammonia spray. Identification was in all cases effected by the use of a marker of authentic gentisic acid.

Representative species of 80 families of Angiosperms were examined by these methods, and positive identifications of gentisic acid were obtained in 73 families. No free gentisic acid was detected in neutral plant extracts; preliminary hydrolysis is essential for the release of the acid from the complex in which it occurs. Studies are in progress on the nature of this complex in tissues of *T. cacao*.

Although gentisic acid was of more frequent occurrence in leaf tissue than caffeic acid, the latter compound is usually present in larger amounts. No correlation was observed between the distribution patterns of gentisic acid and those of other fluorescent phenolic constituents of the leaf extracts.

Although gentisic acid is found in both herbaceous plants and trees, it was notable that in the families included by Hutchinson[6] in the Lignosae, gentisic acid was of more frequent occurrence and tended to be present in larger amounts than was observed in the Herbacae and Monocotylae. It is therefore believed that gentisic acid may be concerned in lignification or in some process associated with it. A similar association has been observed by Bate-Smith in relation to the distribution of leucocyanidins in leaves[7]. Some correlation was also apparent within a family, between the degree of lignification of the genus and the content of gentisic acid present. The relation of gentisic acid to lignification will be discussed in a paper to be published elsewhere.

The widespread occurrence of gentisic acid in plants is also of interest from the point of view of host–parasite relations, since a number of phenolic acids, including protocatechuic acid (3 : 4 dihydroxybenzoic acid), have been shown to confer disease resistance upon certain species of plants. That the content of bound gentisic acid in the surface tissues of the plant may be a factor in the resistance of the plant to fungal attack is, moreover, supported by the observations of Klöpping and van der Kerk[8], who showed that a derivative of gentisic acid from *Populus candicans* possesses highly fungicidal properties *in vitro*.

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Rhizobium Needs Magnesium, not Calcium

In tropical countries there occur abundant examples of legumes that are normally nodulated despite the fact that they grow on acid soils, low in calcium. Observations of some particularly striking examples of this behaviour in the poor 'Wallum' soils of south-east Queensland led to a re-examination of the symbiotic system in the legumes[1]. It was postulated that the tropical legume equipped with cowpea-type *Rhizobium* is adapted to these low-calcium environments because they were the conditions under which the symbiosis evolved, while the clovers and clover type *Rhizobium* are specialized types that have developed calcicole habits. Growth experiments with the bacteria using clays saturated to varying degrees with calcium using McCalla's technique[2] appeared to support the hypothesis, in that strains known to require lime, such as those from lucerne, gave growth in proportion to the degree of calcium saturation of the clay, whereas cowpea-type strains showed relative indifference to the calcium-level. A basic study of the relative calcium requirements of these types was then begun, using a liquid medium in which calcium was supplied in solution, not absorbed on a clay.

To do this a new medium had to be devised. The standard growth medium for *Rhizobium* includes yeast extract which simultaneously supplies nitrogen, growth-factors and minerals, including a significant quantity of calcium. Before a study of calcium requirement could be made it was necessary to prepare a basal medium entirely free from calcium by eliminating yeast extract.

The medium used was constituted as follows:

Disodium hydrogen phosphate	0·45 gm.
Magnesium sulphate	0·1 gm.
Potassium nitrate	0·6 gm.
Ferric chloride	0·01 gm.
Mannitol	10·0 gm.
Thiamin	100 μgm.
Biotin	0·5 μgm.
Distilled water	1,000 gm.

Each ingredient was freed of calcium and all glassware was scrupulously washed with acid. No calcium could be detected by complexometric analysis of the

complete medium. The *p*H of the medium after autoclaving was 7·5 and when 0·02 per cent calcium chloride was added, 6·9.

In this medium 48 strains of *Rhizobium* selected to cover a cross-section of Leguminosae were serially cultured at approximately 14-day intervals for ten generations in comparison with the same medium containing calcium at the normal rate (0·02 per cent calcium chloride). Growth was made in screw-cap tubes containing 10 ml. of medium that was not shaken. All strains made some growth that varied from a trace to 6×10^8/ml. In many cases growth in the series lacking calcium was noticeably superior to that in the parallel series with calcium added. To eliminate the possibility of calcium being obtained from the glass of the culture tubes, transfer of six strains, representing pea, clover, lucerne, cowpea, cassia and acacia, was made from the seventh generation to silica vessels. Serial passage in these vessels to the sixteenth generation was made without loss of vigour.

Mutation, accompanied frequently by loss of infectivity, was high in this medium, but it occurred equally in the series free of calcium and that to which calcium was added.

This experiment showed clearly that *Rhizobium*, whether of clover or cowpea-type, required no calcium for growth. Nevertheless, there remains to be explained the repeated observation that the application of lime to certain soils in which *Rhizobium* will not multiply renders them suitable for growth. One such soil, a krasnozem, was described by Vincent and Waters[3] from Pearce's Creek, near Lismore, New South Wales. Some of this soil was obtained and it was confirmed that, following autoclaving, no multiplication of clover *Rhizobium* was possible in it, but after the addition of calcium hydroxide equivalent to a dressing of 6 tons/acre of lime, populations of $3–5 \times 10^8$/gm. were achieved in seven days. The *p*H of this soil following autoclaving is approximately 5·0 and it could be argued that the application of lime produced bacterial growth either by eliminating acidity or by supplying needed divalent calcium ions. However, when acidity was eliminated by the hydroxides of sodium, potassium and ammonium, no growth occurred. Furthermore, when equivalent quantities of hydroxides of magnesium or barium were applied, they were as effective as calcium in promoting growth. This eliminated Ca^{2+} as the essential factor but pointed to the need for a divalent cation.

From this point a return was made to the synthetic medium which contained magnesium as its only divalent cation. By several simple experiments it was possible to prove that magnesium was the essential element needed by *Rhizobium*. If the magnesium sulphate in the medium was replaced by sodium sulphate no growth occurred. The addition of calcium, strontium or barium had no effect, but the addition of magnesium resulted in immediate growth.

Apparent growth responses to added calcium on soil or clay are explicable by the position occupied by Mg^{2+} in the lyotropic series. Additions of sodium, potassium or ammonium ions will produce no response since these do not displace magnesium. Additions of divalent calcium or barium ions produce growth response because they displace and render available Mg^{2+} for the bacteria.

Following repeated observations during these experiments that added calcium appeared to be actually inhibitory, presumably by antagonizing the uptake of magnesium, the results were applied immediately to the improvement of growth in agar culture medium, which traditionally contains large additions of calcium carbonate or chloride. By eliminating added calcium compounds and strongly augmenting the amount of magnesium sulphate added, considerable improvement of growth of slow-growing types was obtained.

A full account of these experiments will be published elsewhere.

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Field Observations of Midday Closure of Stomata in the Oil Palm, *Elaeis guineensis* Jacq.

During the course of studies on stomatal movement of oil palms under field conditions, some effects of soil moisture on midday closure have been observed. These are of particular interest when examined in the light of the hypothesis of Heath and Orchard[1] on the relationship between leaf temperature and midday stomatal closure.

The technique used was a modification of the infiltration method of Molisch[2], employing a series of *iso*propanol–water mixtures. The results are in good agreement with actual measurements using material fixed by Lloyd's method. All the palms examined were in an area of approximately ¼ acre; they were three years old at the start of this work. Each observation consisted of readings carried out on leaflets from the tip, mid and base of each of six leaves, three on the north and three on the south side of one palm. Each set of three leaves comprised a young, middle-aged and an older, but not senescent, leaf.

Preliminary work in 1956 indicated that midday closure occurred during the latter half of the dry season, which normally extends, with very little or no rain, from November to April, but disappeared with the onset of the rains. During the same period in 1957, midday closure was followed daily. After any appreciable rain, stomatal closure became less severe and remained slight or non-existent until the effect of the rain wore off, and a return to the former state occurred. The extent of closure was correlated with total daily evaporation as measured by a raised tank evaporimeter; after rain a relationship still appeared despite the reduced closure.

To obtain further information on the effect of rain, a comparison was made during the severe dry season of 1958 of the extent of midday closure in two palms, one of which was amply watered. After 33 days the second palm was also watered to show any differences not due to treatment. Air temperature was measured at each observation time

Table 1. The Effect of Watering on Midday Closure of Stomata in the Oil Palm. Palm 1 was Watered throughout and Palm 2 during the Second Period only. The Units of Stomatal Aperture are Arbitrary, see Fig. 1

	Period 1		Period 2	
	Difference in aperture temperature		Difference in aperture temperature	
Palm 2 (unwatered)	3·39	1·50	—	—
Palm 1 (watered)	±0·14	±0·15	0·03	0·10
Palm 2 (watered)	—	—	±0·07	±0·48

by means of a thermometer 75 cm. above ground-level in the shade of the palm. All the readings were taken between 13h. and 13.30h. local apparent time.

The results are presented in Table 1. There is a significant difference between the extent of midday closure in the two palms which disappears when the second palm is watered. The significant difference in air temperature under the two palms also disappears after watering. Each point in Fig. 1 is a mean of eighteen readings on a single palm plotted against the mean temperature under the palm during the observational period. A difference of 3° C. between the air temperature – leaf temperature relationship for the two treatments would account for the observed differences between the watered and unwatered palms. A significant reduction in the temperature of fully exposed leaflets due to watering was demonstrated, but the instrument used was broken before calibration.

Heath and Orchard propose that midday closure of stomata is a temperature effect operating through the carbon dioxide concentration in the intercellular space. The results presented here confirm their observations on onion and coffee, especially as stomatal closure in the range 30–35° C. is so marked. No leaf temperatures are known, but the effect of watering in reducing air temperature is very clear. The observed lag period between watering and the resulting drop in air temperature indicates that the latter is not due to evaporation at soil-level. Although increasing leaf temperature may cause stomatal closure, it is clear that midday closure of stomata may also be influenced by water strain, whether this operates directly on aperture, with a consequent temperature change due to altered transpiration, or whether, as seems likely in view of Heath and Orchard's work, transpiration affects leaf temperature, which determines stomatal aperture. There would appear to be some justification for regarding midday closure of stomata as a sensitive indication of water stress[3,4].

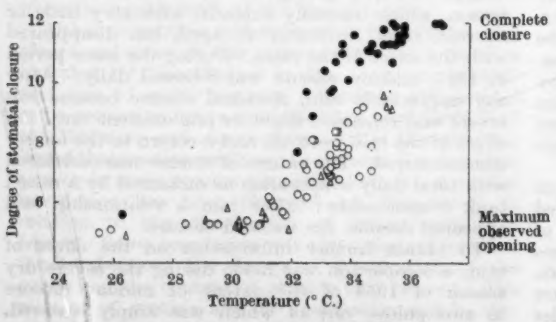

Fig. 1. Extent of midday stomatal closure in watered and unwatered palms plotted against air temperature under the palms. ●, Palm 2 unwatered; ○, palm 1 watered; △, palm 2 watered

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Light Microscopic and Electron Microscopic Observations on the Dividing Walls of Cells in *Chara* spp.

In the simple as well as in the higher plants (Thallophyta and Cornophyta) the structure of cell walls exhibits a great variety of characteristics of partly microscopic, partly submicroscopic order of magnitude. These structural peculiarities, which may be characteristic of wider relationships or even of species, are closely connected with the particular functions of the cell. By our investigations, carried out on the cell walls of the genus and species *Chara*, for example, *Chara foetida* A. Br., *Ch. contraria* A. Br., *Ch. hispida* L., *Tolypellopsis stelligera* Mig., *Nitella* sp., *Tolypella intricata* (Trentep.) or Leonhardi, light was cast upon the connexion existing between structure and function.

All material was first treated with dilute hydrochloric acid, and, after thorough washing, with tri-ethanolamine[1], making the dividing walls easily accessible, since the treatment causes the cells to fall apart. Both bulging end-walls of the large internodes were then severed, cut into small pieces and placed on a collodion film. Preparations were shadowed with palladium at a shadowing angle of 15–35°.

In all species examined the end walls of the internodial cells were found to be perforated (Fig. 1). The pores do not arrange themselves regularly; a peculiar system in which mostly two, sometimes three, structural units of different orders of size are desirable (Fig. 2). The largest structural units on the bulging end-wall seem to be regular, polygonal areas produced by the walls of the adhering smaller nodial cells which are in contact. These areas are visible even under the ordinary stereo microscope. We believe these big polygonal areas to be pore fields as they are perforated all over. The distribution of the perforations, however, is irregular; they occur in larger and smaller aggregations, thus forming secondary structural units. These are surrounded in the pore fields by islets consisting of a dense network of cellulose microfibrils. The islets are interconnected by extensions and thus form a lattice delimiting smaller or larger spaces in which a pore cluster is usually contained, the mesh of microfibrils being densely perforated. Less frequently, the lattice encloses regular, triangular or quadrangular spaces and exhibits thinner strands running diagonally, formed by still smaller pore clusters aggregated within the larger ones and thus representing structural units of the third grade (Fig. 2).

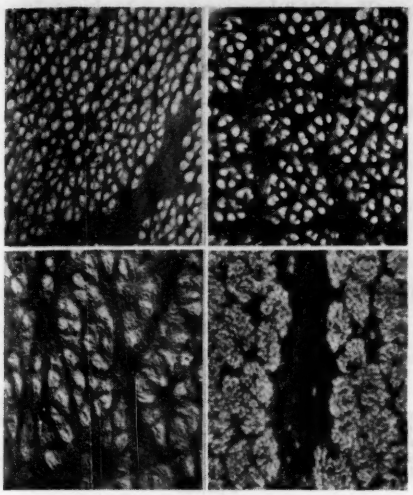

Fig. 1. Detail from the end-wall of the axial internode of *Chara contraria*. (Electron micrograph, × 3,400)

Fig. 2. Pore clusters in the end-wall of the axial internode of *Chara contraria*. (Electron micrograph, × 4,000)

Fig. 3. Detail from the end-wall of the axial internode of *Chara hispida*. (Electron micrograph, × 4,000)

Fig. 4. Detail from the end-wall of the axial internode of *Chara contraria* in the phase contrast microscope. (× 750)

The average diameter of the pores is 400 mμ, their shape is a more or less distorted circle, surrounded by a densely woven texture of cellulose microfibrils. At times the opposite apertures of the pores do not coincide, the pores presumably being oblique. The various species show differences as to the form, dimensions and arrangement of the pores (Fig. 3).

The islets and the perforations mentioned above being within the range of the light microscope, their microscopic examination was also undertaken. Under the phase-contrast microscope not only the network surrounding the pore clusters but also the larger pores themselves were easily observable (Fig. 4).

With the help of easily recognizable islets, identical spots could sometimes be photographed in the light microscope and in the electron microscope. It was proved by further examinations that the walls communicating with the end-wall of the axial internode of the smaller nodial cells, as well as the contiguous walls of the internodial and nodial cortex cells were also perforated. The side-walls of the axial internode and of the internodial cortex cells, however, do not have a similar porous structure[2].

It may be assumed with certainty that the pores in question are the crossing points of the cytoplasmic threads connecting the contiguous cells, the so-called plasmodesmata. These are responsible for the unity in the physiological functioning of the alga and are analogous to the porous structure recognized in the cell wall, more precisely in the pits of the cell wall of higher plants[3-6]. It is due probably to the extraordinary size of the cells that the pores of various *Chara* sp. have dimensions well within the range of the light microscope. The pore fields are of the order of mm.[2] and the network probably serves to strengthen the comparatively large, but exceedingly thin, porous membrane.

Full details of this work will be published in the near future. We wish to thank Mrs. T. Tóth for the excellent technical help she has given.

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Effect of Storage Conditions on the Incidence of *Gloeosporium* Rots of Apple Fruits

SAMPLES of Cox's Orange Pippin obtained from various parts of south-east England were held in cold (37° F.) and in gas (39° F., 3 per cent oxygen and 5 per cent carbon dioxide) storage for periods of approximately 3½ months and 4½ months respectively. On removal from storage, the samples were examined for rots caused by *Gloeosporium* spp., but owing to lack of time the causal species on each rot was not identified. It was noted during 1955–56 and 1957–58 and, to less marked extent, in 1956–57 also, that in certain samples the percentage of apples recorded as damaged by these rots was less after gas storage than after cold storage, despite the higher temperature and longer period of storage, whereas with other samples the converse occurred.

A preliminary examination of seventeen samples after gas storage in 1957–58 indicated that the different behaviour of samples in this respect might be caused by a difference in the predominating species of *Gloeosporium*. Larger sub-samples, of up to 25 apples, were therefore taken from four samples that showed decreased rotting in gas store and from four that showed increased rotting, each sample being from a distinct orchard. The fungus causing each rot was identified by its spores, apples with non-sporing rots being kept at room temperature in polyethylene bags until as many of the rots as possible had been identified. The results are presented in Table 1.

G. album usually develops in storage more slowly than *G. perennans*, and this might make it more susceptible to the concentrations of carbon dioxide and oxygen reached some days after the store is

Table 1. ROTTING OF SAMPLES IN COLD AND IN GAS STORE, IN RELATION TO PERCENTAGE OF ROTS DUE TO *Gloeosporium album*

Origin of sample	Percentage of apples infected with *Gloeosporium* spp.		No. of rots due to :		Percentage of rots due to *G. album*
	Cold store	Gas store	*G. perennans*	*G. album*	
Eynsford	76	35	25	49	64
East Malling	34	9	4	26	87
Norwich	24	17	5	10	67
Beccles	57	23	4	94	96
Chelmsford	8	16	18	3	14
Canterbury	27	41	84	4	5
Burlingham	29	48	98	7	7
Marden	12	32	70	—	—

closed. Alternatively, its late development in store may be linked with an advanced stage of maturity of the apple and therefore make it more likely to be affected by a treatment such as gas storage that delays maturity.

Quite apart from the potential economic value of this finding, if confirmed, the results suggest that there are important differences in the biology and mode of parasitism of these two species of *Gloeosporium*.

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Terpene Formation in Pine from Mevalonic Acid

Recent studies have demonstrated that mevalonic acid is a key intermediate in the biosynthesis of animal sterols[1]. Studies of the origin of triterpenes and carotenoids in soya bean and fungi indicate that these compounds may also be formed from acetate through mevalonic acid[2]. Although the incorporation of atmospheric carbon dioxide into pine terpenes has been demonstrated[3], studies on the nature of the pathway by which these compounds originate have not been reported. In the present work, seedlings of *Pinus attenuata* were found to incorporate acetate-2-^{14}C and mevalonic acid-2-^{14}C into the monoterpenes. The turpentine of this pine is 98 per cent α-pinene[4].

Shoot tips were cut from actively growing 2-year-old seedlings of *P. attenuata*. These shoot tips (about 3 gm.) were placed in cone-shaped vials containing 1 ml. of the radioactive substrate in 0·01 *M* potassium phosphate buffer, *p*H 5·9. The vials, with the base of the shoot tips immersed, were sealed with 'Parafilm' wax paper to prevent evaporation. The tops were then exposed to a 100-watt incandescent lamp and air was blown across them to induce rapid transpiration and uptake of the substrate. Sufficient water was added to the vials to keep the stem bases submerged during the 8 hr. they were allowed to metabolize the absorbed solutions. On removal the shoots were frozen in liquid nitrogen, wrapped in aluminium foil and stored at −10° C. until extracted. Extraction was by homogenization in ethyl ether with mortar, pestle and sand. The samples were extracted until radioactivity was absent from the ether extract. Finally, the homogenized tissue was shaken in a closed Erlenmeyer flask on a mechanical shaker for 3 hr. with four changes of ether. This ether was combined with the earlier extract and the combined volume (about 200 ml.) was reduced to 20 ml. by distillation at 38° C. This concentrate was passed through a column (70 mm. high, 15 mm. diameter) packed with basic alumina oxide (Woelm) and 'Darco' decolorizing charcoal. The column was washed with approximately 100 ml. of ether yielding a colourless eluant containing a minimum of acid residue. The ether eluant was concentrated by distillation to 0·5 ml. and pure α-pinene (0·2 ml.) was added to the ether concentrate. The α-pinene was then separated from a portion of this mixture by gas-liquid-partition chromatography with helium as the mobile phase and 'Carbowax' 1540 on diatomaceous earth as the stationary phase. The α-pinene, fraction *A*, was collected at 120° C. (Fig. 1). The column temperature was then increased to 190° C. while helium was permitted to flow through for 30 min. to flush the column. Fraction *A* was then

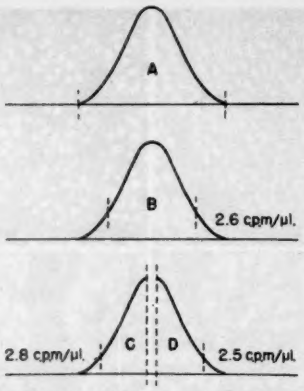

Fig. 1. Specific activity in carbon-14 of α-pinene fractions characterized by gas chromatography. α-Pinene was extracted in ethyl ether from shoot tips of *Pinus attenuata* incubated for 8 hr. with 8·8 × 10^5 c.p.m. mevalonic acid-2-^{14}C. Counted by liquid scintillation in 0·5 per cent 2,5-diphenyloxazole in toluene (ref. 6). Specific activity expressed as c.p.m./μl. of α-pinene

resubjected to chromatography at 120° C. and fraction *B* isolated. Fraction *B* was again chromatographed and separated into two fractions, *C* and *D*. The infra-red spectra of the material isolated in fractions *B*, *C* and *D* were identical with that of pure α-pinene; the specific activities of the pinene in each of these fractions was found to be constant (Fig. 1).

The nitrosochloride was then prepared from another portion of the final ether extract containing carrier α-pinene. The specific activities present in the crystals isolated after three and four recrystallizations are given in Table 1. From this evidence we may conclude that the carbon-14 derived from mevalonic acid is incorporated into α-pinene in pine. Complete details of this study will be published elsewhere.

The amount of radioactivity recovered in the α-pinene of the shoot rips was approximately 0·02 per cent after 8 hr. incubation. When slices of *P. attenuata* were incubated for 2 hr. with mevalonic acid-2-^{14}C, 0·5 per cent of the activity was recovered in α-pinene. In similar experiments with acetate-2-^{14}C, approximately 0·01 per cent was incorporated in the α-pinene fraction of whole shoot tips. However, acetate can be metabolized many ways in pine tissues[5]. It would therefore be erroneous to conclude from these preliminary studies with intact tissues that mevalonic acid is incorporated in preference to acetate.

I wish to thank Drs. N. T. Mirov, P. K. Stumpf and R. Lemmon of the University of California, Berkeley, for the use of laboratory facilities and for constant encouragement in these studies; appreciation is also expressed to the Fundamental Research Group at Merck and Co., Rahway, New Jersey, for

Table 1. Specific Activity in Carbon-14 of α-Pinene Nitrosochloride.

Nitrosochloride of α-pinene formed from concentrated ethyl ether extract of shoot tips of *Pinus attenuata* incubated for 8 hr. with mevalonic 8·8 × 10^5 c.p.m. acid-2-^{14}C. Carrier non-radioactive α-pinene was added to the ether solution. The nitrosochloride was dissolved in toluene and counted by liquid scintillation as in Fig. 1
Melting point, 105–108° C.

Times recrystallized	(mgm.)	(c.p.m.)	Specific activity (c.p.m./mgm.)
3	2·2	165	75
4	1·2	95	79

the initial sample of mevalonic acid-2-^{14}C in November 1956.

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Effect of Simulated Field Deposits of Gamma-BHC and Dieldrin on Susceptible, Hybrid and Resistant Strains of *Anopheles gambiae*, Giles

SINCE the first recognition of resistance to dieldrin in *Anopheles gambiae* in Northern Nigeria[1], laboratory studies have shown the degree of resistance to this insecticide to be of a very high order—some 800 times—and that cross-resistance to gamma-BHC is involved, though of a comparatively low order—some 30 times[2]. This type of resistance has further been shown to be monofactorially inherited, the hybrid being intermediate in its resistance—some 30 times to dieldrin and only 8 times to gamma-BHC[3]. The resistant strain remains susceptible to DDT, though there has always been some doubt as to the efficiency of this insecticide in controlling malaria transmitted by *A. gambiae* because of the irritant effect of the insecticide and the consequent low mortalities achieved[4].

A series of observations has now been made to determine the effect of simulated field applications of commercial formulations of gamma-BHC and dieldrin on susceptible, hybrid and resistant strains of *A. gambiae* derived from colonies maintained at the Ross Institute of Tropical Hygiene, London. The main purpose of the experiment was to see if these applications would produce any mortality among the hybrid and resistant mosquitoes and, if so, for how long. In addition, the persistence of the two insecticides at similar dosages on sorptive mud blocks has been compared.

Blocks of a highly sorptive mud from Babati in Tanganyika, 3 in. in diameter and ½ in. thick, were kindly prepared and sprayed with insecticide by A. B. Hadaway and F. Barlow of the Colonial Insecticide Research Unit, Porton Down.

Gamma-BHC was applied at 25 and 50 mgm./ft.^{2} and dieldrin at 50 mgm./ft.^{2}, using in both cases 50 per cent dispersible powders. The test method used was for the most part similar to that employed by Hadaway[5]. A 'Perspex' exposure chamber was placed vertically against the treated surface of each mud block and approximately 20 unfed adult mosquitoes were transferred to the chamber from a collecting tube. The experiments were conducted in a constant temperature and humidity room (80° F., 75 per cent relative humidity). The mud blocks were stored between tests under these same conditions and the same blocks used for tests at successive dates. The exposure period of mosquitoes to the blocks ranged from 30 min. to 7 hr. according to the age of the residue. After exposure the mosquitoes were lightly anæsthetized with chloroform vapour and removed to net-covered paper cups where they were fed with 10 per cent glucose solution and held for 24 hr., at the end of which time the mortality was determined. Both male and female mosquitoes were exposed, and though in general the former showed a higher mortality than the latter, the combined mortality has been given in the final rendering of the results. As a control to the experiment mosquitoes were similarly exposed to an untreated mud block.

The results of these exposures up to 8 weeks after treatment of the mud blocks are given in Table 1. It can be seen that all three treatments give very high mortalities with susceptible *A. gambiae* throughout the two-month period when an exposure of one half-hour is made. Such high mortalities have in fact continued with both gamma-BHC and dieldrin at 50 mgm./ft.^{2} to the time of writing (13 weeks) with the same exposure time, though the lower dosage of gamma-BHC now only gives a mortality of about 40 per cent.

Table 1. PERCENTAGE MORTALITIES AMONG MALE AND UNFED FEMALE, SUSCEPTIBLE, HYBRID AND RESISTANT *A. gambiae* EXPOSED FOR VARYING TIMES TO RESIDUES OF GAMMA-BHC AND DIELDRIN OF VARYING AGES

Strain	Insecticide (mgm./ft.^{2})	Exposure period (hr.)	Age of residue: 1 day	1½–2 weeks	3 weeks	6 weeks	8 weeks
Susceptible	Gamma-BHC 25	½	100	93	—	89	95
	Gamma-BHC 50	½	100	100	—	100	100
	Dieldrin 50	½	96	—	—	96	96
Hybrid	Gamma-BHC 25	½	100	69	11	0	—
		1½	—	—	—	66	4
		2	—	—	—	—	62
		3	—	—	—	—	60
		4	—	—	—	—	100
	Gamma-BHC 50	½	100	100	61	0	—
		1	—	—	100	83	39
		1½	—	—	—	100	67
		2	—	—	—	—	96
		3	—	—	—	—	100
	Dieldrin 50	1	94	47	5	6	—
		3	—	—	—	—	4
		7	—	—	—	—	32
Resistant	Gamma-BHC 25	½	45	—	—	—	—
		1	87	5	—	0	—
		7	—	—	—	—	24
	Gamma-BHC 50	½	88	—	—	—	—
		1	100	57	—	0	—
		3	—	—	—	—	20
		4	—	—	—	—	64
		7	—	—	—	—	84
	Dieldrin 50	1	5	0	—	7	—
		2	—	—	—	0	—
		7	—	—	—	—	0
Susceptible	Control	½	—	—	—	0	0
		1	0	16*	—	—	—
Hybrid	Control	½	—	—	0	—	—
		1	—	—	0	0	—
		2	—	—	—	0	—
		3	—	—	—	—	0
		4	—	—	—	—	0
		7	—	—	—	—	0

* Over-anæsthetized.

Fresh deposits of gamma-BHC gave complete mortalities of hybrid *A. gambiae* after exposure of ½ hr., and the higher dosage of gamma-BHC continued to give this mortality with the same exposure time up to 2 weeks after treatment. Dieldrin in the fresh state also killed most hybrids though exposure of 1 hr. was necessary. With ageing of the deposits longer and longer exposures were necessary to produce significant kills. After 8 weeks, 4 hr. exposure was necessary to kill all hybrids on the lower dosage of gamma-BHC and 7 hr. exposure to dieldrin only resulted in a third of the hybrids dying. The higher dosage of gamma-BHC continued to produce high mortality at much shorter exposure times.

The resistant *A. gambiae*, as might be expected, proved much more difficult to kill. In the fresh state both dosages of gamma-BHC gave high kills at quite short exposure times, but after 8 weeks 7 hr. exposure was necessary on the higher dosage to give a high mortality rate. Dieldrin never at any time gave satisfactory kills at the exposures given.

These results indicate that a persistent kill of hybrid *A. gambiae* by gamma-BHC with a consequent delay in the appearance of the resistant strain may be expected where this insecticide is used. However, it should be pointed out that this method of exposure in a small confined space may give rise to artificially high mortalities because of the accumulation of insecticide in the vapour state, a condition less likely to arise in Nature. It should also be pointed out that a resistant strain of the species did arise in Kano, Nigeria, as a result of the use of gamma-BHC, if not over a short period, then certainly over a comparatively small area[6].

The current conviction that two types of resistance occur in anopheline mosquitoes, one to DDT and the other to the dieldrin–BHC group of insecticides, and that the possibility of the two types occurring in one and the same population of the same species is very remote, has led to a consideration of the possible use of mixtures of insecticides for preventing the appearance of resistant strains. The choice would of necessity at present lie between DDT and gamma-BHC and DDT and dieldrin. In view of the marked irritant effect of DDT the chances of a mosquito escaping unharmed from a mixture would be greater where dieldrin is involved, owing to the lower toxicity of this insecticide compared with gamma-BHC. The results of the present experiments would further favour the choice of gamma-BHC in the mixture and indicate that a higher dosage than the customary 25 mgm./ft.^{2} will produce a persistence more in line with that of dieldrin at 50 mgm./ft.^{2}.

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Radioassay of Carbon-14 in Aqueous Solutions using a Liquid Scintillation Spectrometer

Most compounds of biochemical interest have only limited solubility in organic solvents, and the useful fluors described to date have only limited solubility in aqueous solutions. This basic incompatibility has limited the application of liquid scintillation counting methods in many biochemical tracer studies. The method described below permits direct counting of aqueous solutions (or alcoholic solutions). In addition, it permits quantitative recovery of the sample in its original form for further analyses, often an important advantage when the quantity of sample available is limited.

The method is based on the principle of using a two-phase system: a solid phase consisting of a multitude of small, clear particles (plastic or glass) into which a fluor has been incorporated and a liquid phase containing the radioactive mixture to be assayed.

Preliminary experiments were carried out using small cylindrical filaments of 'Pilot Scintillator *B*' 3·2 cm. long and 0·043 cm. in diameter, which had been originally designed for use by physicists in particle-tracking experiments. A bundle of these filaments was packed tightly into a section of glass tubing (0·8 cm. × 4·4 cm.) and aliquots of a standard solution of aqueous sodium carbonate-^{14}C (National Bureau of Standards reference source), containing 1,280 disintegrations/sec./ml., were allowed to seep into the bundle by capillary action. The tube was placed upright in a Wheaton Glass Company counting vial and counted in a Packard 'Tricarb' scintillation spectrometer. As shown in Fig. 1 (inset), the corrected count-rate increased linearly with increasing volume of sample. The efficiency was 29 per cent. When the vial was allowed to stand in the refrigerated counting chamber for several hours, the count-rate was observed to rise markedly as the solution froze. Thawing returned the count-rate to the original value. Quick freezing, on the other hand, did not change the count-rate significantly. This rise in count-rate on slow freezing is attributable to separation of the solute phase so as to concentrate it in the vicinity of the beads. For routine use

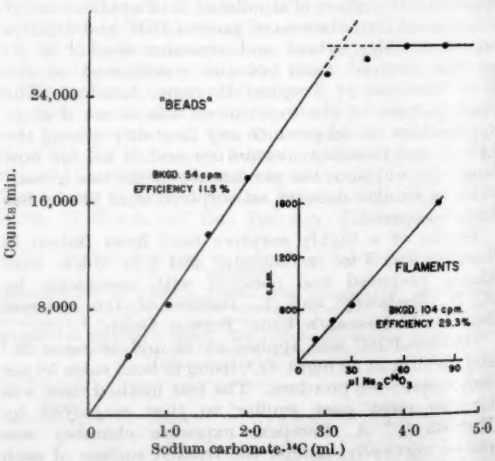

Fig. 1

aqueous samples should be counted above their freezing point. Because the filaments were very tightly packed, it was possible to rinse out the labelled solution without disturbing their packing, and, after rinsing with alcohol and drying, re-use them. A limitation of this method is that the geometry of the system makes it difficult to add an internal standard and be certain of complete mixing. Quenching correction could be determined by adding internal standard to a second aliquot and counting it separately. The hand-packing procedure is tedious and impractical, but if uniformly packed vials of filaments become available commercially, the use of this system might be feasible. A more practical system for routine use would employ uniform spherical beads of plastic scintillator.

The feasibility of the latter approach was explored by cutting the cylindrical filament into very short (1-2 mm.) segments. 4·4 gm. of these 'beads' (approximately 7·7 ml. in volume) were placed in a counting vial and successive aliquots of the aqueous radioactive sodium carbonate standard were added. The count-rate increased in proportion to sample size up to about 3·0 ml. (Fig. 1) and the absolute efficiency was 11·5 per cent. The count-rate then dropped off and reached a plateau as would be expected. With an excess of liquid sample added to a measured quantity of scintillator beads, the observed count-rate is directly proportional to the specific radioactivity of the solution in counts/min./ml. independent of the exact volume added, since the interstices are then completely filled with the labelled solution. This is quite analogous to the counting of solid samples at infinite thickness. It should be pointed out that the density of the plastic used here (1·02) was such that the 'beads' did not pack solidly and this accounts for some of the deviation from linearity seen in Fig. 1. In addition, of course, the simulated 'beads' cut by hand for this purpose were not uniform and did not pack homogeneously. Still, the large number of particles used appears to have resulted in reasonably reproducible average packing. Repeated counts on an aqueous solution of l-lysine-^{14}C, shaken up between counts, were 1,838, 1,773, 1,728 and 1,755 counts/min. Addition of water reduced the observed count-rate in proportion to the dilution of the original radioactive solution, demonstrating completeness of mixing and further validating the direct relation between count-rate and specific radioactivity in counts/min./ml.

Finally, it was shown that a correction for quenching can be accurately made in the usual way by adding a known amount of radioactivity in a minute volume, mixing and recording the increment in count-rate. If a constant quantity of 'beads' is used and an excess of radioactive solution, this internal standard correction also corrects for volume and makes it unnecessary to measure accurately the volume of solution being assayed, provided it completely covers the 'beads'.

The plastic scintillator used in this work, 'Pilot Scintillator B', containing diphenylstilbene, was supplied by Pilot Chemicals, Inc., Watertown, Mass., and I thank Mr. Mark Hyman, of that firm, for providing this material. Uniform short cylindrical segments will soon become available for distribution.

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Stability of Metal Complexes with Salicylic Acid and Related Substances

SALICYLIC acid and its derivatives can form chelate compounds with metal ions. Unfortunately, the paucity of data on stability constants in aqueous solutions[1] has prevented assessment of the biological significance of this property in the outstanding anti-rheumatic and anti-fungal actions of these substances, as well as in their toxic action to man in higher dosage.

Stability constants are now reported for the chelation of some cations with salicylic acid and some related substances. In near-neutral solutions the carboxyl-group of salicylic acid is almost completely ionized, so that simple complex formation with a metal ion would not lead to the considerable liberation of hydrogen ions found, and hence the metal ion must displace the hydrogen ion from the phenolic group. The resulting change in the pH of the solution enabled Bjerrum's potentiometric method[2] to be used to obtain the stability constants of the divalent cations. Where permissible, appropriate mathematical simplifications of the calculations were made[3]. In many instances hydrolysis or precipitation prevented direct determination of $\log K_2$, so for all except cupric and ferric ion, $\log K_2$ was obtained by subtracting $\log K_1$ from $\log K_1K_2$ (found at $\bar{n} = 1$). Constants for ferric complexes were calculated from the dependence on pH of oxidation-reduction potentials[4]. All solutions were titrated under nitrogen. Sodium perchlorate or perchloric acid was added to maintain an ionic strength of 0·15, thus approximating isotonicity with blood. All metals were added as perchlorates except cadmium (sulphate) and manganese (chloride). For all ligands except salicylaldehyde the ionization constant of the phenolic group was measured spectrophotometrically either in sodium hydroxide solution, using known activity coefficients[5] to calculate hydrogen ion concentrations, or in buffer solutions of known pH. All other ionization constants were determined by potentiometric titration.

Reliable ionization constants could not be obtained for salicylamide (which hydrolysed rapidly in alkaline solutions) or for the catechol anion (which oxidized very readily in air). The rough estimate of $pK_2 = 13$ for catechol was based on potentiometric titration under nitrogen.

The present value of $\log K_1$ for the copper salicylic acid complex confirms Babko's[6] figure, but $\log K_2$ is much higher than previously reported[6]. The stability constants of the ferric complexes of salicylic and sulphosalicylic acids are consistent with published[7] data and are much greater than for the corresponding copper complexes.

Stabilities of the complexes of the divalent metals conform to the Irving–Williams series[10]: Mn < Fe < Co < Ni < Cu > Zn. The rather small increment in passing from manganese to nickel, with a greater increase from nickel to copper, is similar to results for other ligands in which bonding of the metal occurs with two oxygen atoms[11].

Table 1 shows that, of the o-substituted phenols examined, both salicylic and 5-sulphosalicylic acid are powerful chelating agents. However, to compare their chelating abilities under biological conditions with those of other ligands it is necessary to calculate from these results the distribution of selected cations between equal ligand concentrations at the same pH. This has been done in Table 2 for approximately neutral solutions of salicylic acid, sulpho-

Table 1. Stability Constants of Some Metal Complexes, in Aqueous Solution at 20° C.

Unless otherwise indicated, ligand concentrations were 0·05 *M* and metal ion concentrations were 0·005 *M*

Cation	Ligand	Log K_1	Log K_2
Mn^{++}	Salicylic acid	5·90	3·9[a]
Fe^{++}	Salicylic acid	6·55[b]	4·7[a]
Co^{++}	Salicylic acid	6·75	4·7[a]
Ni^{++}	Salicylic acid	6·95	4·8[a]
Cu^{++}	Salicylic acid	10·60	7·85
Zn^{++}	Salicylic acid	6·85	pptn.
Cd^{++}	Salicylic acid	5·55	pptn.
Fe^{+++} (0·001 *M*)	Salicylic acid (0·01 *M*)	16·35	11·90
Mn^{++}	5-Sulphosalicylic acid	5·10	2·90
Fe^{++}	5-Sulphosalicylic acid	5·90	< 4·0
Co^{++}	5-Sulphosalicylic acid	6·00	3·60
Ni^{++}	5-Sulphosalicylic acid	6·30	3·90
Cu^{++}	5-Sulphosalicylic acid	9·50	6·80
Zn^{++}	5-Sulphosalicylic acid	6·05	< 4·6
Cd^{++}	5-Sulphosalicylic acid	4·65	pptn.
Fe^{+++} (0·001 *M*)	5-Sulphosalicylic acid (0·01 *M*)	14·60	10·55
Cu^{++} (0·0025 *M*)	2-Hydroxyacetophenone (0·005 *M*)	6·75	5·70
Cu^{++} (0·0025 *M*)	Methyl salicylate (0·003 *M*)	5·90	
Cu^{++}	Salicylaldehyde (0·02 *M*)	5·75	pptn.
Cu^{++} (0·0025 *M*)	Salicylamide (0·005 *M*)	−3·40[c]	−4·50[d]
Cu^{++}	Catechol	1·25[c]	0·65[d]
Fe^{++}	Glycine (for comparison)	4·3[c]	3·5[c]
Cu^{++}	Glycine (for comparison)	8·5[c]	6·9[c]
Fe^{+++}	Glycine (for comparison)	10·0[c]	
Fe^{++}	Oxine (for comparison)	8·0[c]	7·0[c]
Cu^{++}	Oxine (for comparison)	12·2[c]	11·2[c]
Fe^{+++}	Oxine (for comparison)	12·3[7]	11·3[7]

(*a*) Obtained at ligand concentration 0·15 *M*.
(*b*) In 0·1 *M* KCl.
(*c*) For $Cu^{++} + HL \rightleftharpoons CuL + H^+$.
(*d*) For $CuL + HL \rightleftharpoons CuL_2 + H^+$.

Ionization Constants for Ionic Strengths of 0·10–0·15

Ligand	*pK*	
Salicylic acid	2·98,	13·61
5-Sulphosalicylic acid	2·62,	11·95
2-Hydroxyacetophenone	10·06	
Methyl salicylate	9·87	
Salicylaldehyde	8·34	
Catechol	9·43, ~	13
Glycine ($I = 0·01$) (ref. 6)	2·24,	9·85
Oxine ($I = 0·01$) (ref 7)	5·13,	9·89

Table 2. Distribution of Cations among Ligands, in Neutral Solution

	Salicylic acid	5-Sulphosalicylic acid	Glycine	Oxine
Cu^{++}	1	3·5	45	200,000
Fe^{++}	1	10	30	150,000
Fe^{+++}	400	300	1	200

salicylic acid, glycine and oxine (8-hydroxyquinoline). Glycine is a typical amino-acid with which salicylic acid and its derivatives would have to compete for metals in the mammalian body, while oxine resembles salicylic acid in being a powerful fungicide.

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Determination of Strychnine in Nux Vomica by Paper Chromatography

Most methods used for the estimation of strychnine in nux vomica lead to gross inaccuracies due to brucine interference. Even oxidation of brucine with nitric acid under optimal condition (5 min. at 60° C.) leads to errors due to partial destruction of strychnine[1]. Gravimetric estimations also require amounts greater than 0·1 gm. in the sample[1].

Paper chromatography has also been applied, but most of the methods have difficulty in separating brucine from strychnine. Dušinský and Tyllová[2] have overcome this difficulty by using nitric acid to oxidize brucine to *o*-brucichinone. This method probably suffers from errors outlined by de la Vega and Del Pozo[1].

These can be obviated by using a simplified method. The aqueous sample was made alkaline with sodium hydroxide and the alkaloids were extracted with chloroform. The chloroform was then evaporated in a water-bath. The resulting alkaloids were dissolved in a measured volume of chloroform and aliquots of this extract, containing 60–20 γ strychnine, were spotted on to Whatman No. 1 chromatography paper and developed overnight with *n*-butanol/*n*-propanol/0·05 *N* hydrochloric acid (1 : 2 : 1 v/v) at 20° C. by the ascending technique. The paper was dried and photographed in the ultra-violet to reveal the spots. The paper was then cut and eluted with water. The strychnine hydrochloride was estimated using a Beckman spectrophotometer model *DU* at $\lambda_{max.}$ 255 mμ [3].

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Isolation of Piperonylic Acid from *Ocotea pretiosa*

As part of work on the chemical constituents of Brazilian Lauraceae[1], we examined the benzene extractives (2·4 per cent) of the wood of *Ocotea pretiosa* (Nees) Mez, the sassafras tree of the State of Santa Catarina producing essential oil (chiefly safrole). Through extraction with aqueous bicarbonate solution a solid (comprising 0·03 per cent of the wood) was isolated and purified by crystallization from chloroform and vacuum sublimation. White crystals were obtained, melting point 227–29° C. (Kofler block). Determination of the melting point of a mixture and infra-red spectral comparison with an authentic sample established its identity with piperonylic acid.

Piperonylic acid is, apparently, quite rare in Nature. Its known occurrence is restricted to the plant family Lauraceae, where traces of it have been found accompanying relatively much larger amounts of substances containing the piperonyl moiety. Only once before[2] has it been isolated by solvent extraction from a natural source, the coto barks, where it occurs along with 6-piperonyl-α-pyrone (*Aniba coto* Kostermans) and accompanying 6-piperonyl-α-pyrone, 2,4-dimethoxy-6-hydroxy-3′,4′-methylenedioxybenzo-

phenone and 2,4,6-trimethoxy-3′,4′-methylenedioxybenzophenone (*Aniba pseudocoto* Kostermans). Although traces have also been found in steam-entrained camphor oils (accompanying safrole) from several *Cinnamomum* species[3,4], at least once[4] doubt has been expressed as to its actual presence in the plant.

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Shape of Spot and Rate of Sorption in Paper Chromatography

Chromatography is characterized by a dynamic distribution of a substance between a stationary phase and a mobile phase in which diffusion occurs[1]. In the case of a linear sorption isotherm, the exchange reaction between the two phases may be considered to be of the first order.

In spite of this, most theories of chromatography are based on simple equilibrium considerations and diffusion is frequently neglected[1-3]. Although diffusion is considered in a series of papers on sorption-rates by Wicke[4], he considers sorption fronts only and does not apply his analysis to the exchange reaction which occurs in chromatography. In recent papers[5] Giddings has considered the exchange reaction using a discrete model of chromatography, thereby obtaining results similar to ours.

In the present communication we show for a simplified model how the diffusion in the mobile phase and the dynamic character of the exchange process may be taken into account. The random exchanges of a molecule between the adsorbed and the desorbed states interact with the bulk flow in the mobile phase to create a random motion, which is observed macroscopically as an additional diffusion superimposed on the ordinary diffusion in the mobile phase. We shall show that this increase in diffusion in the direction of the flow in the mobile phase may be derived from the general differential equations for the concentrations of adsorbed and desorbed molecules. These equations have been discussed by us in connexion with electrodiffusion[6].

In paper chromatography the spot is often observed to be elongated in the direction of the flow of the developer[1,7]. This fact has not yet to our knowledge been satisfactorily explained. We shall, however, show that for the simple model considered here and in the absence of secondary reactions or transformations on the paper, this may be explained as a consequence of the interaction between the exchange process and the bulk flow. By comparing the dimensions of the spot in the direction of developer flow and at right angles to that direction, it is possible to estimate the values of the rate constants for adsorption and desorption.

Consider a thin layer of a fluid phase streaming in the x-direction with uniform velocity v over a two-dimensional fixed phase. The substance which takes part in the chromatographic process is introduced at zero time at the point $x = y = 0$. We shall assume the initial distribution to be given by a δ-function with the whole amount of substance in the fixed phase. It will further be assumed that we can neglect diffusion in the fixed phase.

If the concentrations of the substance considered are c_1 in the mobile phase and c_2 in the fixed phase, the behaviour of the system, taking into account only motion in the x-direction, may be described by the partial differential equations:

$$\frac{\partial c_1}{\partial t} = D\frac{\partial^2 c_1}{\partial x^2} - v\frac{\partial c_1}{\partial x} - k_1 c_1 + k_2 c_2 \quad (1)$$

$$\frac{\partial c_2}{\partial t} = -k_2 c_2 + k_1 c_1 \quad (2)$$

D is the simple diffusion coefficient, whereas k_1 and k_2 represent the rates of adsorption and desorption, respectively, with respect to the fixed phase and include all effects due to the boundary layer and to diffusion at right angles to the fixed surface. A discussion of the relation between actual reaction-rates at the surface and diffusion towards the surface has been given by Prigogine[8].

These equations are solved by Fourier transforms with respect to x, retaining in the solution of the resulting ordinary differential equations only terms up to the first order in Dz^2, where z is the new independent variable. We obtain:

$$c_1 = \frac{k_2}{2(k_1+k_2)\sqrt{\pi \mathbf{D}_x t}}\left(\exp -\frac{\xi^2}{4\mathbf{D}_x t}\right)[1 + f_1(\xi,t)] \quad (3)$$

$$c_2 = \frac{k_1}{2(k_1+k_2)\sqrt{\pi \mathbf{D}_x t}}\left(\exp -\frac{\xi^2}{4\mathbf{D}_x t}\right)[1 + f_2(\xi,t)] \quad (4)$$

where $\mathbf{D}_x$ represents the 'effective diffusion coefficient' in the x-direction given by:

$$\mathbf{D}_x = \frac{Dk_2}{k_1+k_2} + \frac{k_1 k_2}{(k_1+k_2)^3}v^2 \quad (5)$$

and ξ is a distance co-ordinate the origin of which is moving along with the maximum of concentration, that is,

$$\xi = x - \frac{k_2 v}{k_1+k_2}t \quad (6)$$

The functions $f(\xi,t)$ contain rapidly decaying transient terms.

After a time sufficiently long for the transient terms to become insignificant, the chromatography process in the simplified model considered may thus be described by an ordinary diffusion equation with diffusion constant $\mathbf{D}_x$ and distance co-ordinate ξ.

Following the same procedure as before, it is readily shown that the concentration distribution in the y-direction is given by:

$$c_i = \frac{k_i}{2(k_1+k_2)\sqrt{\pi \mathbf{D}_y t}}\left(\exp -\frac{y^2}{4\mathbf{D}_y t}\right) \quad (7)$$

where

$$D_y = \frac{Dk_2}{k_1 + k_2} \quad (8)$$

The solution of the two-dimensional form in x and y of equations 1 and 2 is the product of the solutions 3 and 7, and it follows that the contour of the spot corresponding to a given concentration is an ellipse the principal axes of which, a_x and a_y, are proportional to the square roots of the effective diffusion coefficients in the x- and y-directions, that is :

$$\frac{a_x}{a_y} = \sqrt{\frac{D_x}{D_y}} \approx 1 + \frac{k_1 v^2}{2D(k_1 + k_2)^3} \quad (9)$$

The present theory thus provides not only a value for the rate of advancement of the spot but also gives an explanation for its shape. Furthermore, by comparing the two axes of the elliptical spot at different velocities, it is possible to deduce the values of the rate constants k_1 and k_2 for adsorption and desorption. More accurate values of the rate constants k_1 and k_2 may be obtained by measuring the rate of spreading of a spot or band at different velocities of developer flow.

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Migration-elution of Zones in an Electrophoresis Column

So far the preparation of electrophoretically defined fractions by means of zone electrophoresis in powder columns has been performed in two separate steps. Electrophoresis has been succeeded by displacement of the solution in the column[1,2] or the entire contents of the column have been removed from the tube and cut in segments from which the substance has been recovered by washing[3,4]. Both methods obviously suffer from a number of drawbacks. The latter technique is rather time-consuming and is especially complicated when applied on a large scale (100-ml. sample or more). The former method is simple in operation but its main disadvantage is its dependence on the filtering properties of the powder. As Kunkel[4] has pointed out, the displacement of zones after electrophoresis will to some extent impair the separation already achieved at the end of the first step.

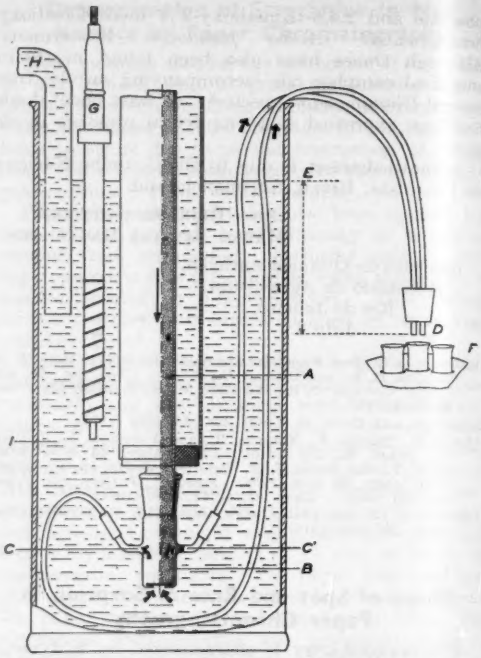

Fig. 1. Arrangement for continuous removal of zones during electrophoresis (see refs. 5 and 6). The electrophoresis column *A* is filled with powder surrounded by a cooling jacket and provided with the short column *B*. In addition to the column the electrode *G* and a tube *H* is dipped into the electrode vessel *I*. The tube *H* connects the buffer in *I* with a buffer reservoir for keeping the liquid-level *E* constant

We have found it possible to remove continuously the zones at a fixed level in the column by a buffer flow operating in the direction opposite to the electrophoretic migration. A simple arrangement for the utilization of this principle is shown in Fig. 1. In column *A* the zones move electrophoretically towards the bottom and pass a thick filter paper and a platinium net at the end of *A*. After entering a second short column *B* attached to *A* they meet a counter current of buffer solution. This causes the displacement of the zones through the side arms *C* and *C'* and through two capillaries the other ends of which are placed above a tube in a fraction collector *F*. The counter flow of liquid, which is determined by the hydrostatic pressure head *ED*, should be adjusted so that it safely exceeds the electrophoretic migration velocity of the fastest moving zone. Too high a rate of flow should be avoided, of course, since it will unduly dilute the samples to be collected. For obvious reasons the liquid flow should be decreased with time if the electric field-strength is kept constant during the experiment. A continuous decrease of the rate of flow proportional to the time should be most convenient.

The side arms may be placed directly at the lower end of *A* or at the upper end of the tube if electrophoretic migration upwards is preferred. However, the arrangement in Fig. 1 makes it possible to fill the tubes *A* and *B* with different anticonvection media. This opens up new fields of application of column electrophoresis. If the tube *A* is filled with a material

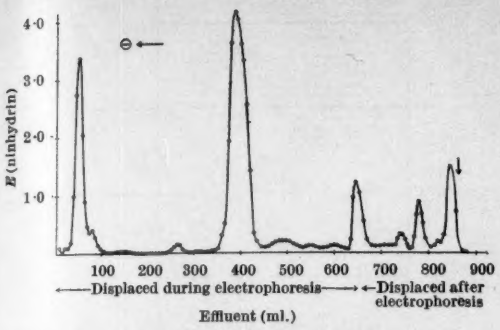

Fig. 2. Electropherogram of a mixture of basic peptides of the posterior lobe of pig pituitary (starting zone marked by the arrow). The sample had been obtained after a number of purification steps, including extraction, precipitation, ion-exchange chromatography and counter-current distribution. The large peak at the effluent volume of 400 ml. corresponds to lysine-vasopressin. When about 640 ml. of effluent had been collected during the electrophoretic run, the electrophoresis was stopped and the substances still remaining in the column were displaced by fresh buffer solution

of good stabilization but poor filtering properties such as a plastic powder or even a gel and if *B* is filled with powder suspension, a continuous displacement of zones should be possible with the retention of a high degree of resolution. Such experiments are now in progress.

Fig. 2 illustrates the result of an application of the method. The experimental details were as follows: 200 mgm. of a mixture of basic peptides from the posterior lobe of pig pituitaries were dissolved in 1 ml. of 0·3 *M* pyridinium acetate solution (*p*H 4·9) containing 0·5 per cent phenol as a preservative. The solution was applied at the top of a cylindrical column 71 cm. long, 2 cm. wide, cooled externally and containing the same buffer stabilized by cellulose powder[7]. The solution was allowed to sink into the column by the force of gravity and was displaced by fresh buffer to a position 2 cm. below the upper surface. The small column *B* was now attached to the lower end of *A* (Fig. 1) and immersed in the buffer of electrode vessel *I*. Liquid connexion with the other electrode vessel was established as described elsewhere[5,6] and the free ends of the capillaries *D* were placed over the fraction collector. The column *A* was cooled by circulating tap water of +10° C. through the cooling jacket. A potential difference of 480 V. between the electrodes resulted in a current of 40 m.amp. After 22 hr. the elution through the capillaries was started while continuing the electrophoresis. One 5·9-ml. fraction was collected every half hour. After 71 fractions had been collected the pressure head *ED* was decreased to yield 3·6 ml./hr. and one fraction was collected hourly. From fractions 115 to 142, 2·3 ml. of eluent an hour was removed from the column. The electrophoresis was now stopped and the material remaining on the column displaced by fresh buffer at a rate of 5·3 ml./hr. One fraction was collected every 5 min. Ninhydrin values according to Moore and Stein[8] were determined on 0·5 ml. aliquots from each sample. The distribution curve is shown in Fig. 2. The large peak in the middle corresponds to lysine-vasopressin.

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Neutron Production and Temperature in ZETA

It is assumed that thermalization and high temperature have been achieved in ZETA[1], although theoretical considerations demand a much longer period than that available for the process to take place[2]. This discrepancy between apparently observed and the theoretically expected thermalization time may be due to unknown processes occurring which speed up the process of thermalization[2]. This explanation, however, does not seem to be valid. Moreover, it is possible to explain most of the observed facts, if one assumes a 'betatron type' of acceleration of deutrons in ZETA, for the following reasons:

(*a*) Theoretical and experimental work[3–5] have shown that under suitable conditions 'run-away electrons' are produced in gas discharges; that is, under certain conditions the cross-section for electron – ion collision is small. This implies that under these conditions the ions can move freely through the electrons with mean free paths much larger than those computed from considerations of plasma density.

(*b*) Using the method of estimating critical velocity for 'run-away electrons'[5] and the equations of Spitzer[4], the critical energy required for ions to be continuously accelerated against ion–ion collision losses is $W = 3{\cdot}2 \times 10^{-14}\, n/V$ electron volts, where n is the number of ions per cm.^{3} and V is the electric field in volts per cm. In ZETA, with $n = 6 \times 10^{13}$ and initial value of $V = 2$ volts per cm., $W = 1$ eV. Thus, even at room temperature there is an appreciable number of ions (about 10^6) out of a total of about 10^{19} ions in ZETA, which could be accelerated. The number of neutrons produced per pulse is also of a similar order; but this may be just a coincidence. The initial adiabatic compression could contribute still further to this number of eligible ions.

Once the ions are in a condition suitable for acceleration, an average field of 1 V./cm. could accelerate the ions to 60 keV. in 0·5 m.sec. or to 240 keV. in 1 m.sec. The azimuthal magnetic field due to 2×10^5 amp. is sufficient to confine ions of even higher energy; but ions of very high energy may not be produced because of the chance of ion loss by ending in nuclear reactions with the large number of relatively stationary ions.

If this mode of ion acceleration is accepted, it is easy to resolve the difficulties in explaining most of the facts observed with ZETA.

(1) Some of the deutrons undergo 'betatron type' of acceleration and gain enough energy in the observed time of about 0·5–1 m.sec. and produce neutrons by colliding with the large number of relatively slow-moving ions, as explained above. The difficulty about thermalization time[5] automatically vanishes.

(2) The anisotropy of neutron energy observed by means of photographic emulsion technique cannot be explained by any process of thermalization; but it is a necessary corollary of this method of acceleration of ions. (This experiment was mentioned during a lecture at the Tata Institute on May 19, 1958, by Dr. T. E. Allibone, to whom this possibility of acceleration of ions in ZETA was briefly mentioned.)

(3) The oxygen and nitrogen atoms in the discharge could gain random energy perpendicular to the discharge axis due to scattering by accelerated deutrons, and this could explain the observed Doppler broadening of their spectral lines when observed perpendicular to the discharge axis. If these ions also acquire a large drift velocity due to the collision of deuterons and the electric field, observation of the spectrum in a direction tangential to the discharge should reveal a Doppler shift as well.

(4) The observed decrease in neutron production with any increase of initial gas pressure could be due to the increase in the critical energy, which will reduce the number of ions eligible for acceleration.

In the light of this explanation, ZETA is a zero energy toroidal accelerator somewhat similar to that proposed by Budker[7]. The ions move in a direction opposite to that of the electrons and behave like a one-dimensional gas which is not in thermodynamic equilibrium and hence not obeying Maxwell's distribution law. Temperature assignment to such a gas has only dubious value. Nevertheless, this does not preclude the possibility of a fusion reactor of the type of ZETA, even though it may never be a truly thermonuclear reactor.

I am grateful to my colleagues, Mr. S. S. Bhave, Dr. A. S. Divatia, Mr. M. K. Mehta and Dr. A. P. Thatte, for stimulating discussions.

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Ductility of Chromium deposited from Molten Chloride Baths

Some time ago[1], we reported that chromium was deposited from certain molten chloride baths as hexagonal crystals which are transformed on cooling to the cubic form, as shown by X-ray examination.

In our further study of ductility in these crystals, we have found that while a low oxygen-level (less than 0·02 per cent) is necessary for ductility, the position of the oxygen in the lattice is also important. This position is, we believe, reflected in electrode potential. With a given oxygen content ductility is always associated with lower electrode potential against pure chromium. The hexagonal crystals show a break in the electrode potential temperature

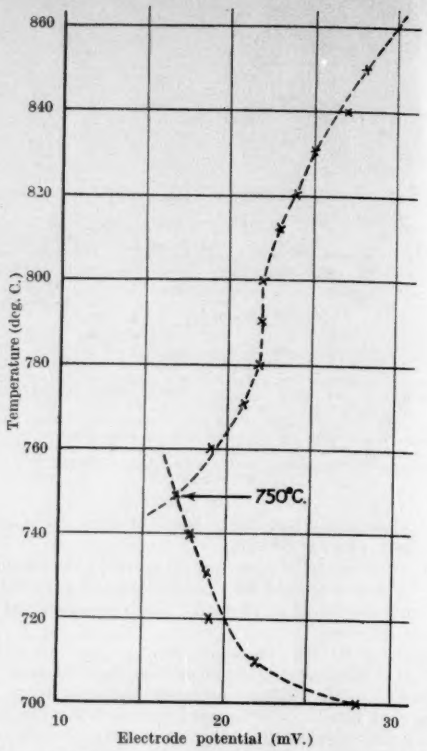

Fig. 1. Cooling curve of our chromium against commercial chromium

curve when measured against ordinary chromium. This is shown in Fig. 1. Ductility is thought to be determined by the mechanism of transformation from the hexagonal to the cubic form, and particularly by the distribution of impurities in the new lattice.

If crystal growth takes place along an uninterrupted screw axis, then the chromium is ductile by deformation along these axes. The photomicrograph (Fig. 2) is of a platelet of chromium from fused salt electrolysis bent 135° and cut and polished on the plane of the original platelet. The etch is electrolytic.

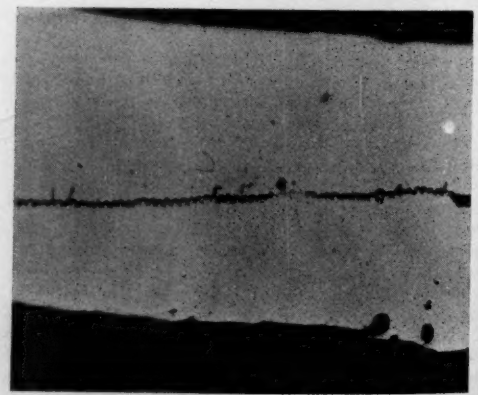

Fig. 2. Platelet of chromium, bent 135° and cut and polished on the plane of the original platelet. (× 75)

Many such screw 'deformations' occur in bending chromium platelets of this kind.

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A Highly Sensitive Chemical Dosimeter for Ionizing Radiation

An aqueous dosimeter capable of measuring radiation doses of less than 100 rads would be an extremely useful tool in the field of radiobiology. Although doses of a few rads can be accurately measured using the resorcinol-stabilized tetrachlorethylene-dye systems[1], such systems where the radiation-induced reaction proceeds by a chain mechanism exhibit certain undesirable characteristics. An alternative to the radiation-induced chain reaction involves the measurement of extremely small amounts of an irradiation product by means of a sensitive analytical technique. Some idea of the extreme sensitivity required is obtained from the fact that a dose of 100 rads will produce approximately 10^{-7} moles of product per litre of solution, assuming a reasonable production-rate of unity for $G_{prod.}$ (that is to say, 1 molecule of product formed per 100 eV. absorbed). An analytical method capable of accurate measurement at such high dilution is spectrophotofluorometry. The production of salicylic acid (which fluoresces in aqueous solution) in irradiated aqueous solutions of benzoic acid seemed to merit investigation, especially because this reaction had been proposed[2] as suitable for chemical dosimetry.

During irradiation a mixture of the three isomeric hydroxybenzoic acids is produced in the ratio of 5 : 2 : 10 for the *o* : *m* : *p* isomers respectively[3]. However, at 400 mμ (exciting wave-length 290 mμ) the fluorescent intensity of the *m* and *p* isomers is small compared with that of salicylic acid and can be neglected. Diphenyl has also been identified as a radiation product, but it does not fluoresce at 400 mμ if excited at 290 mμ. The fluorescent intensity of salicylic acid solutions is a linear function of concentration in the range 10^{-7}–10^{-3} *M*, and is independent of acidity from *p*H 5–11. Since the reproducibility of the measurements was only about ± 10 per cent, presumably because of the fluctuations in the intensity of the light source of the Aminco–Bowman spectrophotofluorometer, each experimental run was calibrated separately in the following manner. The fluorescent intensities of two standard salicylic acid solutions were measured immediately before and immediately after measurements were carried out on a series of irradiated solutions, and the salicylic acid concentrations in the latter solutions were computed using the average value of the 'fluorescence coefficient':

$$\text{Fluorescent coefficient} = \frac{\text{fluorescent intensity of standard}}{\text{[salicylic acid]}}$$

All dose-rates were determined using the ferrous sulphate −0·8 *N* sulphuric acid dosimeter[4]. Corrections for the decreased energy absorption in water were made as described previously[5].

From Fig. 1 it is seen that the concentration of salicylic acid in irradiated 10^{-4} *M* calcium benzoate solutions (*p*H 5) increases in a linear fashion with dose of X- and γ-radiation over the range 0–100 rads, and is independent of dose-rate over the range 4–50 rads/min. For a given dose the fluorescent intensity is almost identical for cobalt-60 and cæsium-137 γ-rays. The rates of salicylic acid production expressed as $G_{Sal.}$ were computed to be:

Incident radiation	Co^{60} γ-rays	Cs^{137} γ-rays	300-kV. peak X-rays	100-kV. peak X-rays
Effective energy (MeV.)	1·25	0·66	0·16	0·05
$G_{Sal.}$	0·63 (15·5)	0·63 (15·5)	0·63 (15·5)	0·52 (13·8±0·5)

The values in parentheses refer to the values of the oxidation yield of the ferrous sulphate dosimeter used in the computation of $G_{Sal.}$

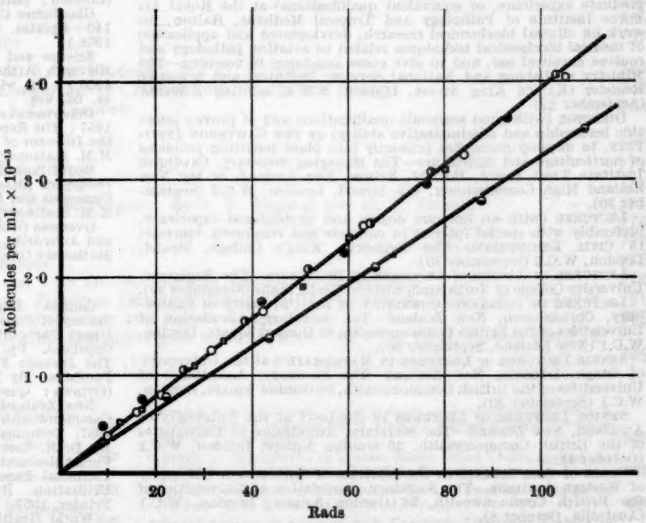

Fig. 1. ◒, 100-kV. peak X-rays (20 rads/min.); □, 300-kV. peak X-rays (17 rads/min.); ●, 600-keV. cæsium-137 γ-rays (4 rads/min.); ○, 1·25-MeV. cobalt-60 γ-rays (20 rads/min.); ◑, 1·25-MeV. cobalt-60 γ-rays (50 rads/min.)

It is interesting to note that the $G_{Sal.}$ values are in reasonable agreement with the value of 0·73 deducible from the results of Weiss and co-workers[3] on the irradiation of $1{\cdot}6 \times 10^{-3}$ *M* solutions of benzoic acid at *p*H 5 with 200-kV. peak X-rays.

These preliminary experiments indicate that doses of electromagnetic radiation in the range 5–100 rads may be accurately measured (about ± 5 per cent) from the fluorescence induced in calcium benzoate solutions. It is hoped to extend these experiments to the measurement of doses below 5 rads.

W. A. Armstrong
D. W. Grant
Defence Research Chemical Laboratories,
Ottawa, Ontario. July 8.

[1] Hine, G. J., and Brownell, G. L., "Radiation Dosimetry", Chapter 8 (Academic Press, Inc., New York, 1956).
[2] Day, M. J., and Stein, G., *Nature*, **164**, 671 (1949).
[3] Loebl, H., Stein, G., and Weiss, J., *J. Chem. Soc.*, 405 (1951).
[4] Weiss, J., Allen, A. O., and Schwarz, H. A., Proc. Int. Conf. Peaceful Uses of Atomic Energy, **14**, 179 (1955).
[5] Grant, D. W., Ph.D. thesis (Leeds, 1957).

FORTHCOMING EVENTS

Monday, September 15

Institute of Metal Finishing (at Northampton Polytechnic, St. John Street, London, E.C.1), at 6.15 p.m.—Prof. Ernst Raub (W. Germany): "Recent Research on Macro- and Micro-Throwing Power".

Wednesday, September 17

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Dr. T. Salomon (Institut Francais du Pétrole): "Harmful Effects of Electrostatic Charges on Machinery and Lubricating Oils".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Senior Research Associate in Upper Atmospheric Physics, for duties including experimental and observational research on the airglow and aurorae—Prof. D. R. Bates, F.R.S., Department of Applied Mathematics, The Queen's University, Belfast (September 20).

Lecturer or Assistant Lecturer (with interests in palæontology, stratigraphy or sedimentology) in Geology at St. Salvator's College—The Joint Clerk to the University Court, College Gate, St. Andrews (September 22).

Senior Scientific Officer (with a first- or second-class honours degree, preferably in biochemistry, and at least three years' post-graduate experience, or equivalent qualifications) at the Royal Air Force Institute of Pathology and Tropical Medicine, Halton, for work on clinical biochemical research, development and application of medical biochemical techniques related to aviation pathology and routine chemical use, and to give some assistance in teaching—The Ministry of Labour and National Service, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting F.594/8A (September 29).

Director (with good academic qualifications and of proven scientific leadership and administrative ability) of the Cawthron Institute, to develop researches primarily into plant nutrition problems of horticulture and agriculture—The Managing Secretary, Cawthron Institute Trust Board, Box 29, Nelson, New Zealand, or the New Zealand High Commissioner, 415 Strand, London, W.C.2 (September 30).

Lecturer (with an honours degree and professional experience, preferably with special interest in concrete and reinforced concrete) in Civil Engineering—The Registrar, King's College, Strand, London, W.C.2 (September 30).

Lecturer or Assistant Lecturer in Economics—The Registrar, University College of North Staffordshire, Keele, Staffs (September 30).

Lecturer in Inorganic Chemistry at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, September 30).

Senior Lecturer or Lecturer in Mathematics at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (September 30).

Senior Lecturer or Lecturer in Geology at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (October 4).

Chair of Soil Science in the Institute of Agriculture, University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 8).

Lecturer in Agricultural Chemistry at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 10).

Junior Lecturer in Botany—The Registrar, Trinity College, Dublin (October 11).

Professor of Mathematics at the University of Tasmania, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (October 13).

Lecturer or Assistant Lecturer (able to teach in comparative physiology) in Zoology at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

Senior Lecturer/Lecturer in Geography at the University of New England, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, November 14).

Senior Lecturer in Anatomy (Gross Anatomy) at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (November 30).

Assistant Conservator of Forests (with a university degree in forestry, and preferably experience or training in surveying) in Nyasaland—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. BCD.61/4/01.

Assistant Lecturer in Mathematics—The Secretary, Royal College of Science and Technology, Glasgow.

Chair of Metallurgy—The Secretary, Royal College of Science and Technology, Glasgow.

Chemist, Scientific Officer grade (graduate with a good first- or second-class honours degree, preferably with a knowledge of high vacuum techniques), to undertake research with radioactive gases involving the separation of mixtures and thermal diffusion of gaseous isotopes—The Group Recruitment Officer, United Kingdom Atomic Energy Authority, Atomic Energy Research Establishment, Didcot, Berks, quoting 1195/34.

Entomologist (with a good honours degree in natural science and some research experience in entomology) with the Department of Agriculture, Trinidad, to carry out research on biology and control of insect pests of coconuts and to co-operate in assessing their importance as vectors of red ring disease—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/38/01.

Lecturer (with a good honours degree in chemistry, or F.R.I.C., preferably with the desire and ability to undertake research) in Inorganic Chemistry, at Coventry Technical College, to teach subject to Grad.R.I.C. standard—The Director of Education, Council Offices, Coventry.

Zoologist (with a good honours degree and an interest in parasitology, preferably with a knowledge of chemistry) in the Helminthology Department—The Head, The Wellcome Laboratories of Tropical Medicine, 183–193 Euston Road, London, N.W.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Scottish Agricultural Improvement Council: Fruit Trials Committee. Report on Fruit Trials in Scotland, 1949–1956. Pp. 38. (Edinburgh: Edinburgh and East of Scotland College of Agriculture; Aberdeen: North of Scotland College of Agriculture; Glasgow: West of Scotland Agricultural College; Invergowrie: Scottish Horticultural Research Institute, 1957.) [227

Animal Behaviour (formerly *The British Journal of Animal Behaviour*), Vol. 6, Numbers 1 and 2 (January–April, 1958). Published twice yearly. Annual subscription 50s.; U.S.A. and Canada 8 dollars. (London: Baillière, Tindall and Cox, Ltd., 1958.) [227

Glasshouse Crops Research Institute. Annual Report, 1956. Pp. 140+4 plates. (Littlehampton: Glasshouse Crops Research Institute, 1958.) [227

Science and the Idea of God. By Prof. C. A. Coulson. (The Eleventh Arthur Stanley Eddington Memorial Lecture, 21 April, 1958.) Pp. vi+51. (Cambridge: At the University Press, 1958.) 4s. 6d. net. [227

Department of Scientific and Industrial Research. Building Research 1957: The Report of the Building Research Board with the Report of the Director of Building Research. Pp. iv+75+16 plates. (London: H.M. Stationery Office, 1958.) 5s. 6d. net. [227

Royal Botanic Gardens, Kew. Kew Bulletin, Additional Series, 1: *Indigofera* (*Microcharis*) in Tropical Africa, with the related genera *Cyanopsis* and *Rhynchotropis*. By J. B. Gillett. Pp. 1+166. (London: H.M. Stationery Office, 1958.) 30s. net. [227

Overseas Geological Surveys: Mineral Resources Division. Gypsum and Anhydrite. By Dr. A. W. Groves. Pp. iv+108. (London: H.M. Stationery Office, 1958.) 7s. 6d. net. [227

Other Countries

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Memoir No. 286: The Arthropod Fauna of the Upper Carboniferous Rocks of the Maritime Provinces. By M. J. Copeland. Pp. v+110 (21 plates). 1.25 dollars. Memoir No. 287: The Jurassic Fernie Group in the Canadian Rocky Mountains and Foothills. By Hans Frebold. Pp. xi+197 (44 plates). 2.75 dollars. (Ottawa: Queen's Printer, 1958.) [227

New Zealand Forest Service: Forest Research Institute. British Commonwealth Forestry Conference, Australia and New Zealand, 1957. Technical Paper No. 14: Timber Preservation in New Zealand. By D. R. Carr. Pp. 19. Technical Paper No. 21: Integration of Forest Research and Forest Products Research. By J. S. Reid. Pp. 8. Technical Paper No. 23: The Importance of Grading in Timber Utilisation. By W. C. Ward. Pp. 16. (Wellington: Government Printer, 1957.) [227

World Health Organization. Technical Report Series. No. 146: Expert Committee on Water Fluoridation—First Report. Pp. 25. 1 Swiss franc; 1s. 9d.; 0.30 dollars. No. 148: Joint FAO/WHO Expert Committee on Brucellosis—Third Report. Pp. 51. 2 Swiss francs; 3s. 6d.; 0.60 dollars. (Geneva: World Health Organization, London: H.M. Stationery Office, 1958.) [227

Western Region of Nigeria. Annual Report on the Forest Administration of the Western Region of Nigeria for the year 1955–56. Pp. ii+34. (Western Region, Nigeria: Government Printer, 1958.) [227

Eesti Loodus, Nr. 1 (Veebruar, 1958). ("Estonian Nature.") Pp. 64+4 tahvel. (Tartu: Academy of Sciences of the Estonian S.S.R., 5 Hariduse St., 1958.) Rbl. 3. [227

U.S. Department of Health, Education and Welfare: Public Health Service. A Directory of Medical and Biological Research Institutes of the U.S.S.R. Compiled by David P. Gelfand. Pp. vi+340. (Public Health Service Publication No. 587.) (Washington, D.C.: Government Printing Office, 1958.) [227

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